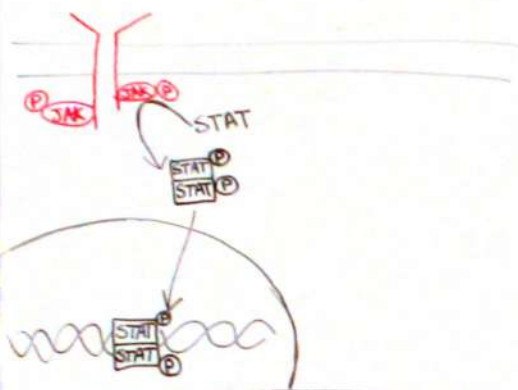
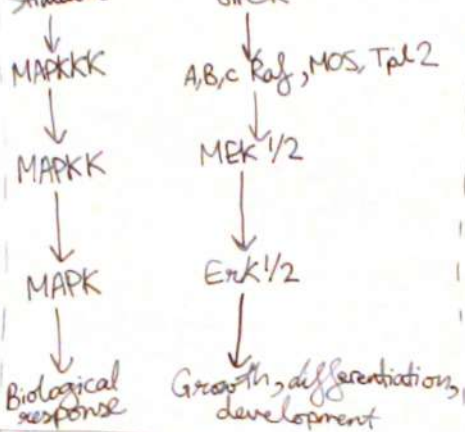


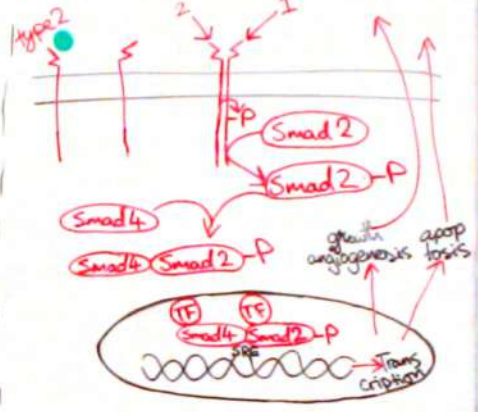
Jak-Stat (non RTK)



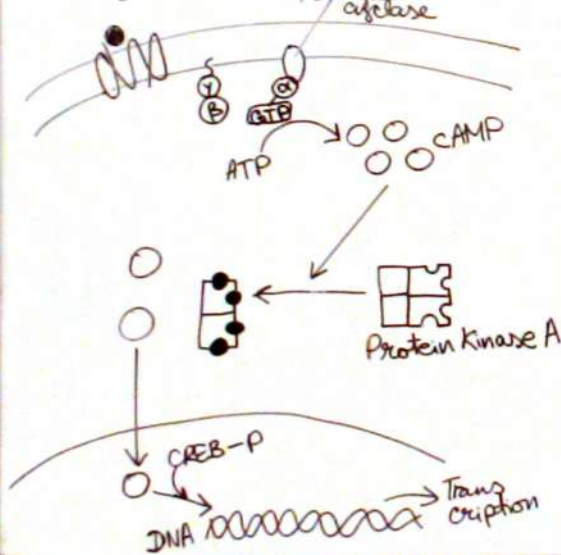
MAPK (RTK or non RTK)



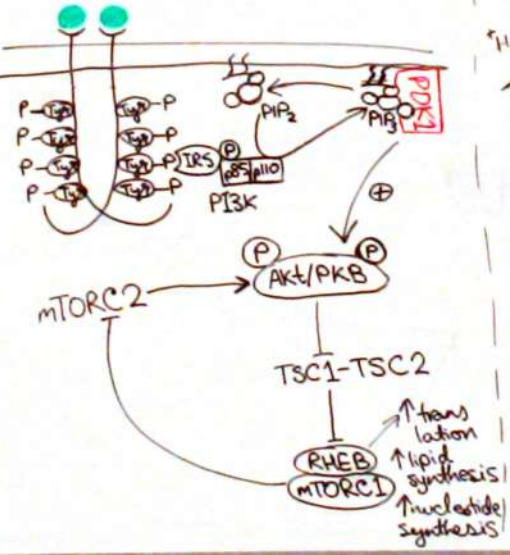
SMAD2/4 Serine/Threonine



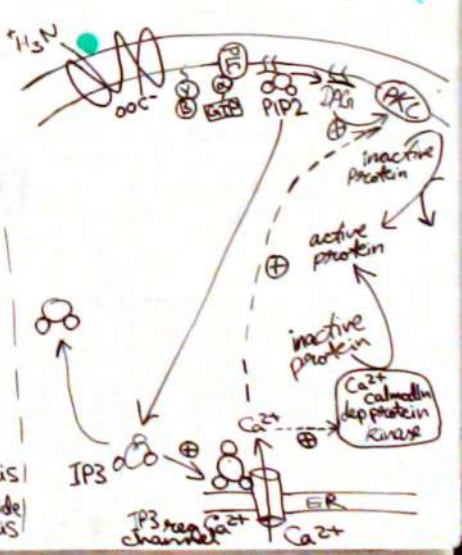
Adenylate cyclase/cAMP GPCR



PI3-mTOR (RTK or non RTK)



PLC-DAG GPCR or RTK

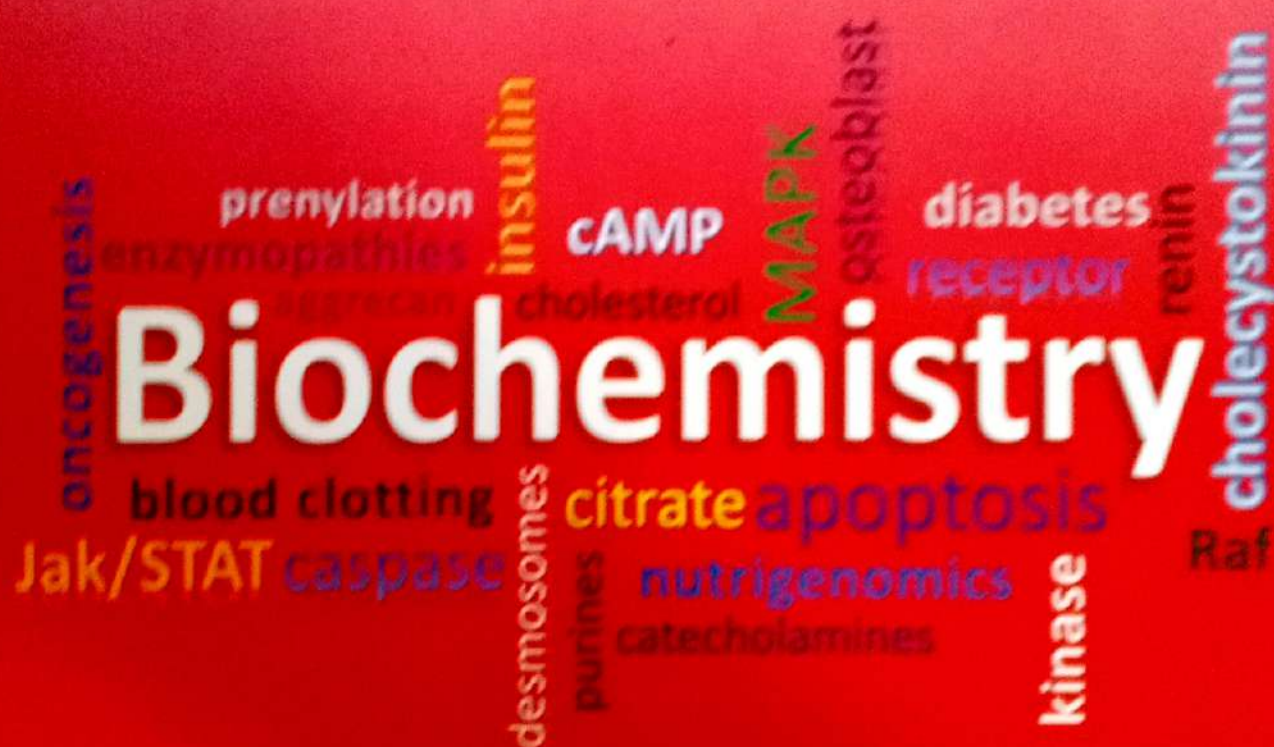


Inotropic Ion Channel mediated	Adenylate Cyclase cAMP	Guanylate Cyclase cGMP	PL3-IP3-DAG
ACh (nicotinic) GABA Glycine Glutamate Serotonin	GPCR $G_{i\alpha}$ or $G_{1\alpha}$ Adrenaline (α_2) (β) $G_{i\alpha}$ $G_{1\alpha}$ Glucagon ACTH TSH Dopamine CRH GHRH FSH, LH Vasopressin (V_2) Somatostatin ACh (M_2, M_4) $G_{i\alpha}$	Guanylate Cyclase (soluble or particulate) NO ANP, BNP, CNP	GPCR ($G_{q\alpha}$) or RTK Angiotensin II Vasopressin (V_1) GnRH ACh (M_1, M_3, M_5) TRH Adrenaline (α_1)
PI3K-mTOR	SMAD2/SMAD4	JAK-STAT	MAPK
RTK Insulin IGF-1 PDGF EGF	Serine, Threonine Kinase receptor (TGF β receptor) TGF β Activins Nodal	non RTK Interferons Interleukins Erythropoietin Growth Hormone	RTK, non RTK Insulin PDGF NGF EGF FGF

Anelia Bivolarska

MEDICAL BIOCHEMISTRY

Part II



Lax book

Plovdiv

2025

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1. Molecular diseases

Molecular diseases **include**:

1. Hemoglobinopathies
2. Enzymopathies
3. Genetic defects of cellular receptors
4. Pharmacogenetic defects

Molecular diseases result from **gene mutations**, most commonly **point** mutations. These mutations affect only **one nucleotide pair**.

Consequences of point mutations in the metabolic pathway

Metabolic disorders due to decreased enzyme activity

Anabolic Pathways

These are referred to as "**deficiency diseases**" due to the inability to form a biologically important product, for example:

- **Glycogen**: The cause of glycogen deficiency disease is the deficiency of the enzyme *glycogen synthase*.
- **Heme**: Defects in the enzymes involved in heme synthesis lead to *porphyrias*.
- **UMP (Uridine monophosphate)**: Defects in the enzymes *orotate phosphoribosyl transferase* and *decarboxylase* result in *orotic aciduria*.

Consequences (Fig. 1-1):

- **Absence or reduced** amount of the *product* and an **increase** in the *substrate*.
- Due to the elimination of the retro-inhibitory effect of the final product, the formation of intermediate metabolites in *side pathways* increases. These metabolites can be *harmless or toxic*. When they are soluble in water, can pass into the urine and blood and serve as **diagnostic indicators**. When they are insoluble, they deposit in tissues and cause **damage**.

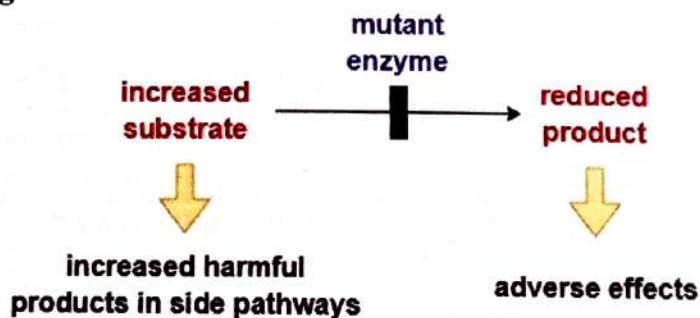


Fig. 1-1 Consequences of an enzyme defect

Catabolic Pathways

These are called "**lysosomal storage diseases**" because **unmetabolized products accumulate in lysosomes** of various organs. Such diseases include *glycogen storage diseases*, *sphingolipidoses*, and *mucopolysaccharidoses*.

- Side pathways can also be activated (a typical example is *phenylketonuria*, where phenylalanine, instead of being converted to tyrosine, is transformed into the so-called "*aromatic keto acids*", which are toxic to neurons).

- A metabolic disorder in one catabolic pathway can affect another metabolic pathway; for example, *glucose-6-phosphatase* deficiency leads to **hyperuricemia** (gout), **hyperalaninemia**, **lactic acidosis**, and **hyperlipidemia** (Fig. 1-2).

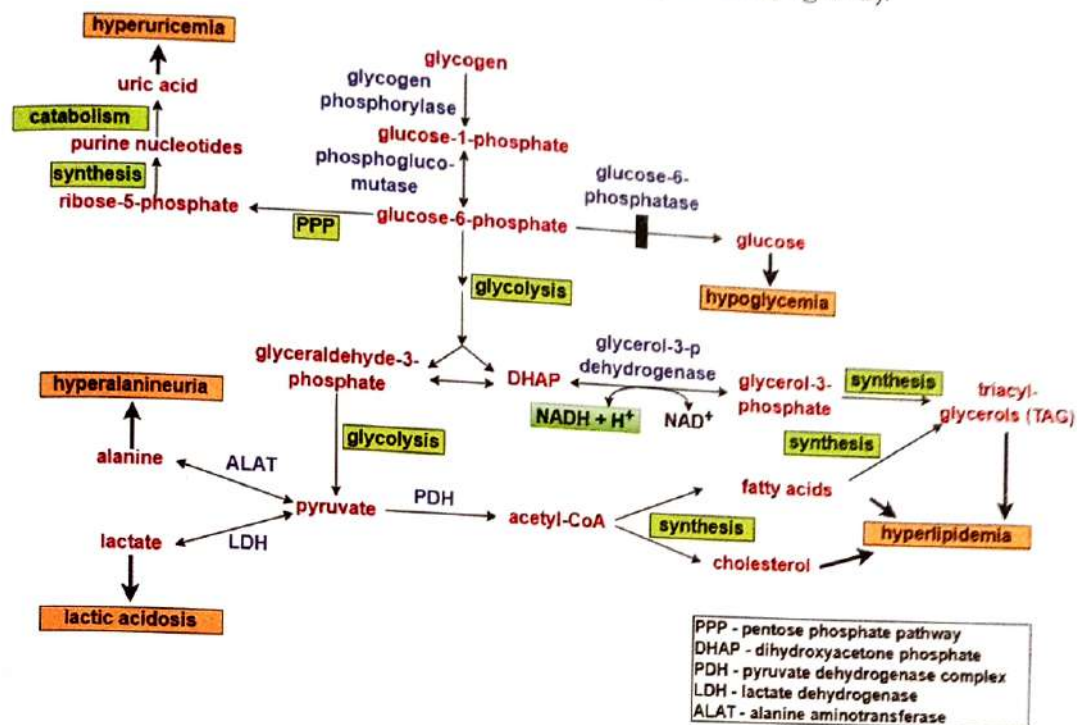


Fig. 1-2 Consequences of *glucose-6-phosphatase* deficiency (Gierke's disease)

Table 1-1 Types of *glycogen storage diseases*

Type	Name/ Disease of:	Enzyme deficiency	Affected organs	Glycogen structure	Glycogen quantity
I	Von Gierke	glucose-6-phosphatase	liver	normal	increased
II	Pompe	α -1,4-glucosidase	all lysosomes	normal	increased
III	Cori	amylo-1,6-glucosidase from the debranching enzyme	all organs	missing or very short external chains	increased
IV	Andersen	debranching enzyme	liver and other organs	very long unbranched chains	normal
V	McArdle	glycogen phosphorylase	muscles	normal	increased
VI	Hers	glycogen phosphorylase	liver	normal	increased
VII	Tarui	phosphofructokinase	muscles	normal	increased
VIII	-	phosphorylase kinase	liver	normal	increased
IX	-	phosphorylase kinase	all organs	normal	increased
0	-	glycogen synthase	liver	normal	reduced

Metabolic disorders due to increased enzyme activity

Anabolic Pathways

Causes:

- Anomalies in enzyme structure.
- Decreased repression/increased derepression when the effect of the final product is eliminated. For example, enzymes before the block in *orotic aciduria*, a defect in the aporepressor protein in *heme synthesis*, leads to *porphyrias*.

Catabolic Pathways

Increased activity can lead to *depletion of metabolites* from the metabolic pathway. For example, *adenosine deaminase deficiency* leads to ATP depletion in erythrocytes and increased hemolysis due to impaired bioenergetics (during purine nucleotide degradation).

Defects affecting enzyme activity regulation

Defects in primary regulation mechanisms

Disorders in allosteric sites

A defect in the allosteric site of the enzyme *phosphofructokinase 1* for citrate removes the limiting negative control on glycolysis, causing an **accumulation of NADH**. The increased NADH hinders the shuttles that transport the hydrogen produced by glycolysis to the mitochondria. The excess NADH reduces dihydroxyacetone phosphate to glycerol-3-phosphate, which is used to synthesize *triacylglycerols*, accumulating as benign nodules under the skin (*lipomas*).

Defects in secondary regulation mechanisms

Disorders in phosphorylation mechanisms

An example is the defect in the *kinase of muscle glycogen phosphorylase*, which phosphorylates and activates *muscle glycogen breakdown*. This is a type of glycogen storage disease.

Defects in tertiary regulation mechanisms

Anomalies in regulatory genes: For example, the inability to synthesize a regulatory gene.

Anomalies in operator genes: Defect in the control of structural genes.

Anomalies in replication control: For example, antibodies against snRNA, which prevents splicing errors, and antibodies against *topoisomerase* in patients with the autoimmune disease *systemic lupus erythematosus*. In this disease, antibodies against nuclear antigens are formed.

Molecular diseases due to defects in repair mechanisms

Repair mechanisms

The role of repair mechanisms is to *remove disturbances* before they are expressed as abnormal proteins. In some cases, this is impossible, and the cell undergoes apoptosis, or the mutations cause disease. In **nucleotide excision repair (NER)**, the enzyme *poly-ADP ribose polymerase* (PARP) is activated, which, with the participation of NAD^+ , performs **ADP-ribosylation** of chromatin proteins. There is a **correlation** between repair intensity and enzyme activity. Enzyme activation can become so high that it depletes cellular NAD^+ reserves. Some molecular diseases involve defects in repair mechanisms themselves. Examples include *xeroderma pigmentosum*, *lymphoreticular neoplasia*, and *Fanconi anemia*.

Diseases due to genetic defects in cellular receptors

Cellular receptors recognize and interact with specific substances (**ligands**) - **hormones, metabolites, drugs**, etc. A genetic defect in a specific type of cellular receptor leads to the inability of the corresponding substance to be absorbed and used by cells (e.g., cholesterol from LDL) or to transmit the corresponding information (if it is a signaling molecule).

Genetic defect of LDL receptors

- Leads to the development of early atherosclerosis.
- The disease is called **familial hypercholesterolemia** and can be due not only to a defect in the *LDL receptor* but also to the *apoprotein B100*, which is recognized by the receptor.

Defects in leptin receptors

- When fat reserves increase, the hormone **leptin** is formed and secreted by adipose tissue, circulating in the blood and passing through the blood-brain barrier.
- Leptin interacts with its *receptor in the hypothalamus*, regulating several hormones produced in the brain (appetite decreases, thermogenesis increases, and fat reserves decrease).
- Multiple defects in leptin's effect in **obesity** are possible.

Mutation in the ryanodine receptor gene

- Leads to defective closure of the receptor on the endoplasmic reticulum during diastole.
- This increases Ca^{2+} leakage from the endoplasmic reticulum, resulting in *ventricular tachycardia*.

Diabetes insipidus

- Caused by unsuccessful folding of a mutated variant of the *V2 gene for the vasopressin (antidiuretic hormone) receptor*.



2. Post-translational regulation of proteins

Proteolytic cleavage

Most proteins undergo proteolytic cleavage after *translation*.

The simplest form is to *remove the initiating methionine*. Many proteins are synthesized as inactive precursors that are activated under appropriate physiological conditions by *limited proteolysis*. Examples include enzymes involved in *digestion, blood clotting and fibrinolysis*. A more complex case of post-translational processing of a *preproprotein* is the cleavage of **pro-opiomelanocortin** (POMC) synthesized in the pituitary gland. It undergoes complex degradation pathways (*proteolytic cleavages*) (Fig. 2-1).

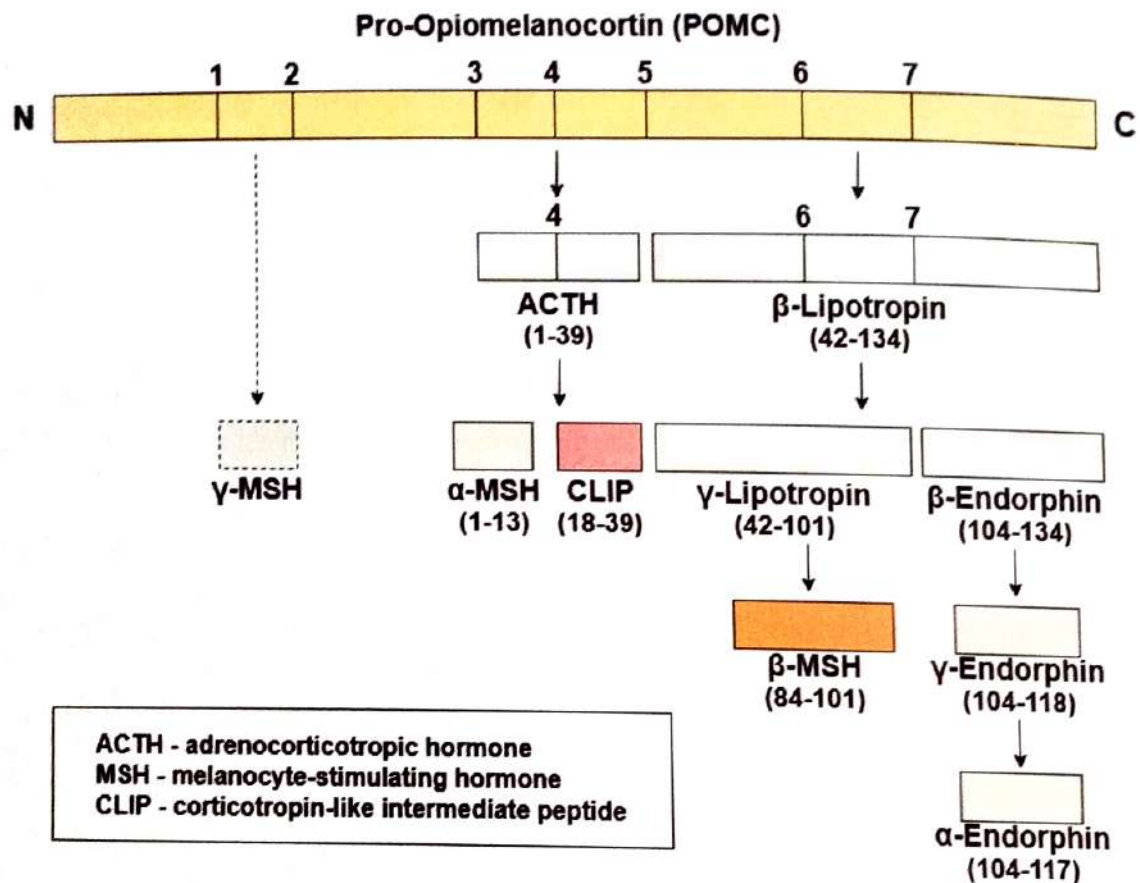
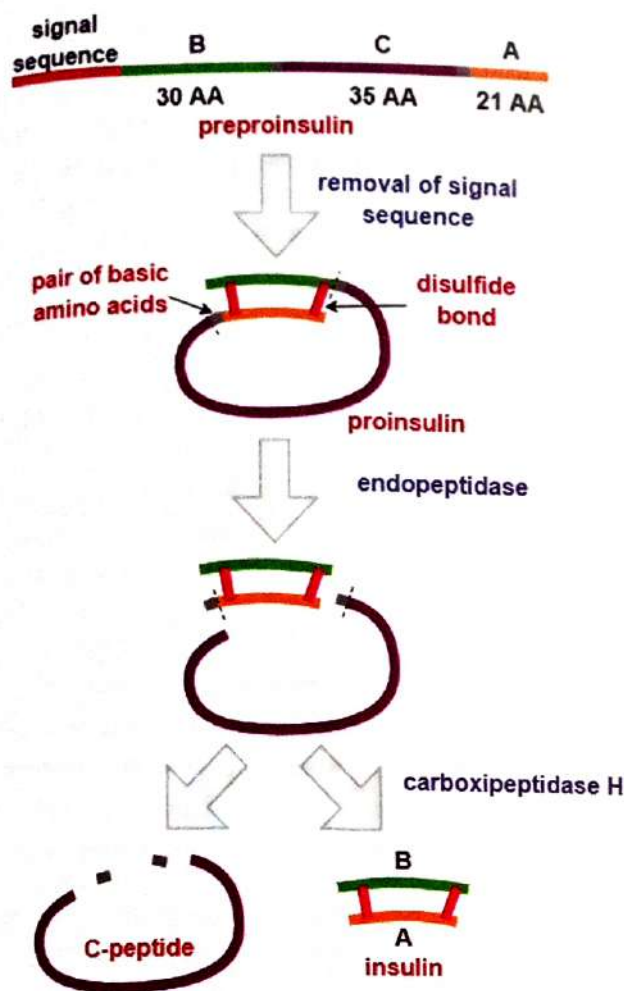


Fig. 2-1 Cleavage of pro-opiomelanocortin

The **POMC gene** is expressed in the anterior and intermediate lobes of the pituitary gland. The **primary protein product** of the POMC gene is a 285 amino acid precursor that can be processed to yield at least **eight peptides**, depending on the site of synthesis and the stimuli that lead to their production.



Insulin

Preproinsulin → *proinsulin* → *insulin*

After the cleavage of the 24-amino acid *signal peptide*, the protein acquires the conformation of proinsulin. Proinsulin is further cleaved into active insulin, which consists of two peptide chains linked by disulfide bridges (**A and B chains**), with an equimolar amount of **C-peptide** being released (Fig. 2-2).

Fig. 2-2 Cleavage of preproinsulin

Coagulation pathways (see Fig. 15-14)

Blood clotting is the process of *stopping bleeding*, involving the vascular endothelium, blood cellular elements, and plasma factors.

The entire coagulation system represents a *stepwise cascade of reactions* (mostly proteolytic) that follow one another until the final phase of **fibrin formation**.

Modification of synthesized proteins by methylation, acetylation, myristoylation and prenylation

Approximately 20,000 genes produce ~100,000 proteins through *alternative splicing*, and these proteins undergo significant *post-translational modifications*, such as the addition of carbohydrate, phosphate, methyl groups, or lipids.

Acetylation

Many proteins undergo N-terminal modification after synthesis. In most cases, the *initiating methionine* is hydrolyzed, and an *acetyl group* is added to the new N-terminal amino acid. **Acetyl-CoA** is the donor of the acetyl group for these reactions, and the enzymes are called *N-acetyltransferases*.

Histone deacetylation results in **chromatin condensation** and **transcriptional block**.

Prenylation

Prenylation refers to the addition of a **15-carbon farnesyl group** or a **20-carbon geranylgeranyl group** to acceptor proteins. These groups are isoprenoid compounds derived from the *cholesterol* biosynthesis pathway. Isoprenoid groups attach to *cysteine* residues at the carboxyl terminus of proteins via a thioester bond. For example, the "**anchoring**" of *Ras* to the cell membrane requires prior *palmitoylation (acylation)* in the C-terminal region and *farnesylation* of cysteine residues.

Prenylated proteins are of significant pharmaceutical interest:

- ✓ **The immune role** of many prenylated proteins is the basis for some of the *anti-inflammatory* actions of **statins**, which are drugs that inhibit cholesterol synthesis, thereby reducing the synthesis of farnesyl pyrophosphate and geranylgeranyl pyrophosphate, and thus decreasing the extent of the inflammatory process.
- ✓ **Prenyltransferase inhibitors can be used to:**
 - suppress the malignant activity of **oncogenic Ras proteins**;
 - act as potential therapeutics against certain diseases, including **cancer, progeria, aging, parasitic diseases, bacterial and viral infections.**

Phosphorylation

Post-translational phosphorylation is one of the **most common protein modifications**. Most phosphorylations occur as a mechanism for regulating the biological activity of the protein and are therefore **transient (reversible)**.

- Significant examples are phosphorylations in glycogen metabolism in response to a signal from **glucagon** secreted by the pancreas. Phosphorylation of **glycogen synthase** inhibits its activity, while phosphorylation of **glycogen phosphorylase** increases its enzymatic activity. (see Medical Biochemistry Part I Fig. 1-34 and 1-35).
- Enzymes that phosphorylate proteins are called *protein kinases*, and those that remove phosphate group from the proteins are called *protein phosphatases*.

Serine, threonine, and tyrosine are amino acids subject to phosphorylation. Accordingly, kinases that phosphorylate these sites are called **serine/threonine kinases, tyrosine kinases, and dual-specificity kinases**.

Sulphation

Sulfate modification occurs at **tyrosine** residues in proteins such as *fibrinogen and secretory proteins* (e.g., gastrin). The universal sulfate donor is **3'-phosphoadenosine-5'-phosphosulfate (PAPS)**. Since sulfate is necessary for biological activity, it is *continuously* added and not used for regulatory modification like tyrosine phosphorylation.

Vitamin-dependent modification

Vitamin C-dependent modifications

1. Acts as a cofactor in the hydroxylation of proline and lysine in collagen synthesis with enzymes **prolyl hydroxylase** and **lysyl hydroxylase** (Fig. 2-4).
2. It takes part in the amidation at the C-terminus of proteins at **glycine**. Several peptide hormones such as **oxytocin and vasopressin** have C-terminal amidation.

Myristoylation

Myristic acid is a saturated fatty acid (C₁₄:0) often covalently attached to the N-terminus of a **glycine** residue in cytoplasmic proteins associated with the cell membrane. The donor for this modification is **myristoyl-CoA**. This modification allows the modified protein to **associate with cell membranes**.

Example: The catalytic subunit of *cAMP*-dependent protein kinase A (AMPK) is myristoylated.

Methylation

Post-translational protein methylation occurs at *nitrogen* and *oxygen* atoms. The active methyl donor is **S-adenosylmethionine (SAM)**.

1. **N-methylation:** The most common methylations occur at the α -amino group of lysine residues, also at the *imidazole ring* of histidine, the *guanidino group* of arginine, and the *amides* of glutamate and aspartate. N-methylation is a permanent change, and no enzymes in mammals are known to remove the methyl group.
2. **Methylation of oxygen:** The carboxyl group of **glutamate** and **aspartate** forms methyl esters.
3. **Thiol group methylation:** The thiol group of **cysteine** in proteins can be methylated.
4. **Histone methylation in DNA:** *Inhibits transcriptional activity*. DNA methylation is an "epigenetic switch" regulating the balance between "open" and "closed" chromatin forms by altering DNA-protein interactions (Fig. 2-3).

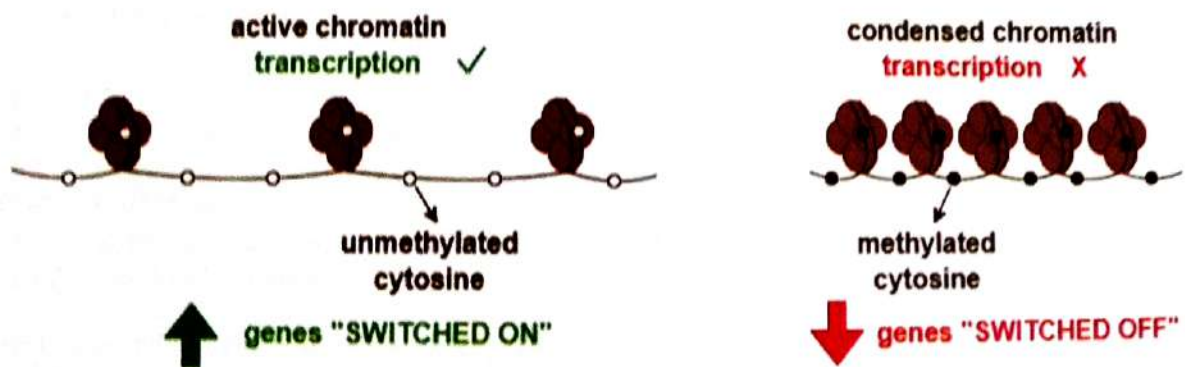
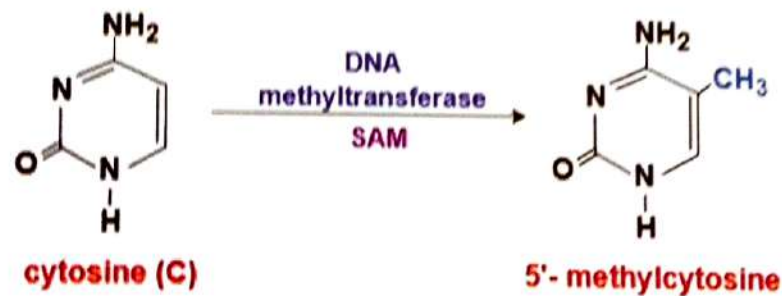


Fig. 2-3 DNA methylation

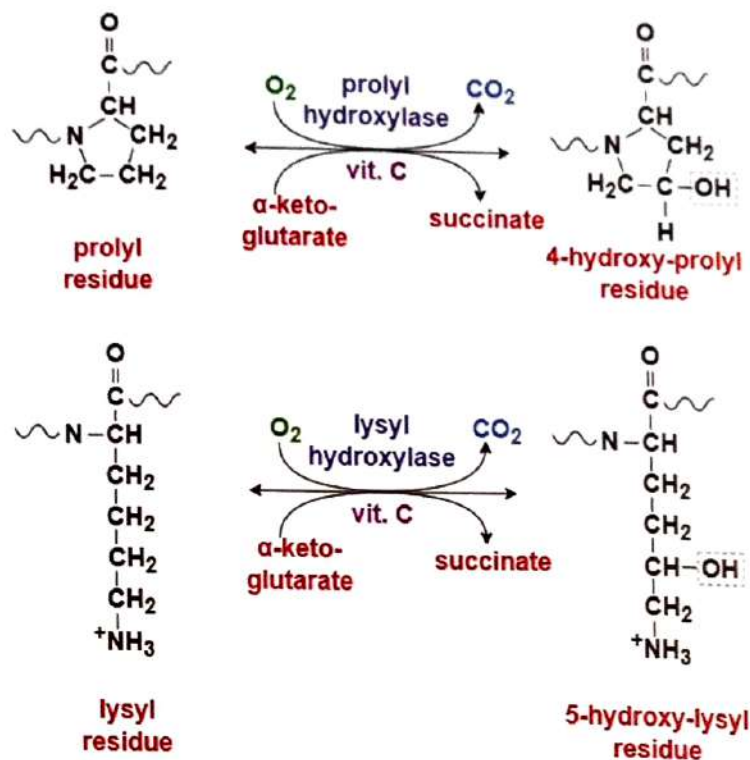


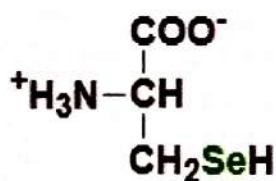
Fig. 2-4 Proline and lysine hydroxylation

Vitamin K-dependent modifications (see Fig. 15-13)

Carboxylation of **glutamate residues** into **GLA residues** (γ -carboxy glutamate) occurs in some of the clotting factors. The presence of GLA residues allows the protein to **chelate calcium ions**. The coumarin anticoagulants, such as **warfarin** and **dicumarol** act by inhibiting this carboxylation reaction. *Vitamin K* is a cofactor of carboxylases in amino acid metabolism.

Selenoproteins

Selenium is a trace element and part of enzymes involved in redox reactions, such as *glutathione peroxidase*, *iodothyronine deiodinase*, and *thioredoxin reductase*. Selenium in these selenoproteins is incorporated as the unique amino acid *selenocysteine* (Fig. 2-5) during translation.



Selenocysteine incorporation occurs during translation, where selenocysteine tRNA is loaded with serine and then enzymatically selenylated to produce *selenocysteinyl-tRNA*.

Fig. 2-5 Selenocysteine

Ubiquitin and the targeting of proteins for degradation

Endogenous protein degradation

Tissue proteins are in a **dynamic state** of continuous synthesis and degradation. Approximately 3-5% of cellular proteins are degraded and synthesized **daily**. Cellular processes are controlled not only by transcription and translation but also by degradation processes.

Proteolytic cleavage

The half-life of different proteins is *strictly regulated*, ranging from hours to days, months, or years, after which they are removed by proteolytic degradation. There are two methods of degradation:

1. By **lysosomal enzymes**
2. In the **proteasome** by binding to ubiquitin (**ubiquitination**)

Proteases and peptidases break down **proteins into amino acids**.

The relative sensibility of proteins to degradation is expressed as the **half-life ($t_{1/2}$)**, the time required for the concentration of a given protein to decrease half of the original concentration.

- The half-life of liver proteins ranges from 30 minutes to over 150 hours.
- Typical "housekeeping" enzymes have $t_{1/2}$ greater than 100 hours.
- In contrast, many key regulatory enzymes (*HMG-CoA reductase*, *ALA synthase*) have $t_{1/2}$ of 0.5–2 hours.

Diseases related to ubiquitination modifications

Several pathological conditions are at least partially due to mutations in motifs related to the recognition of substrates subject to ubiquitination, as well as in enzymes involved in the ubiquitination mechanism:

1. Diseases resulting from *loss of function due to mutations in the ubiquitin enzyme system or target protein*, leading to stabilization of the protein that should normally be degraded.
2. Another group involves mutations leading to *abnormal or accelerated degradation of target proteins*. The most obvious disease that can be expected from defective ubiquitination is *cancer*. Indeed, defective ubiquitin-mediated degradation of growth-promoting proteins such as **fos**, **myc**, and **src** has been observed in cancers.

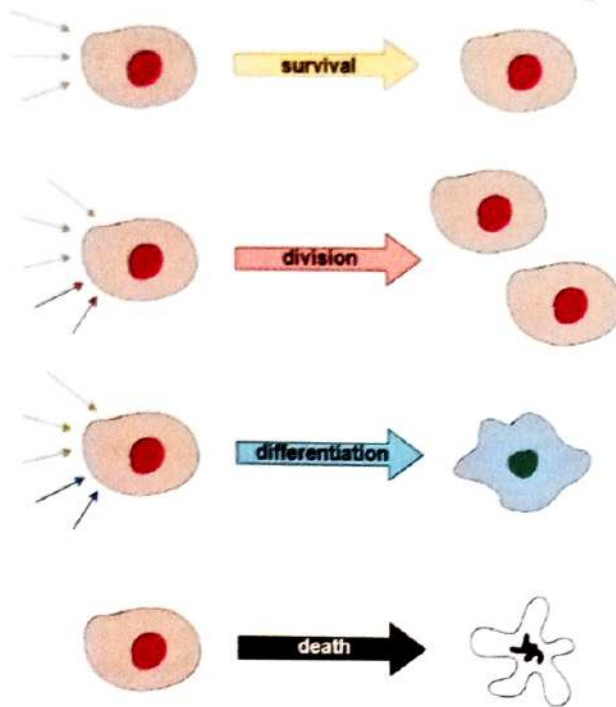
Additionally, defective ubiquitination has been linked to neurodegenerative diseases such as *Parkinson's disease*, *Alzheimer's disease*, and *amyotrophic lateral sclerosis*.



3. Intercellular interactions and signal transduction

Signal transduction is the *transfer of information from the external environment into the cell*. Extracellular signals (also called **first messengers** or mediators) bind to **cellular receptors** and initiate a signaling pathway that leads to a **biological response**.

Cell metabolism and fate depend on multiple extracellular signals (Fig. 3-1).



Each cell expresses a set of receptors that allow it to respond to the corresponding set of signaling molecules produced by other cells. These signals work in combinations that regulate cell behavior.

An individual cell requires multiple signals for survival (*yellow arrows*) and additional signals for cell division (*red arrows*) or differentiation (*blue arrows*).

If deprived of appropriate survival signals (growth factors), the cell will “commit suicide” known as programmed cell death or apoptosis (*black arrow*).

Fig. 3-1 Fate of the cell

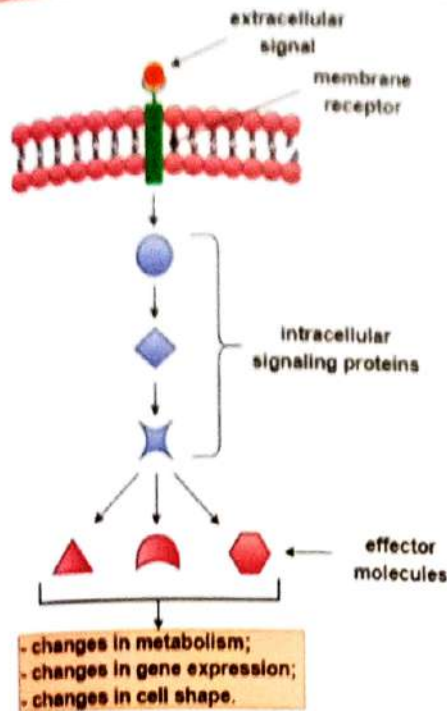
Methods of cell signal transmission

- **endocrine signaling** - extracellular signals are hormones produced in *endocrine glands* or cells, which are transported via the *bloodstream* to the target cell.
- **autocrine signaling** - the cell controls *its own activity* by secreting a signal *outside* the cell, which is then recognized by receptors on the *same* cell.
- **paracrine signaling** - extracellular signal acts on *neighboring cells*;
- **intracrine signaling** - involves the action of *growth factors, cytokines, and steroid hormones* *within* the cell. Before puberty, extragonadal tissues produce estradiol in a paracrine and intracrine manner, but not in an endocrine manner.
- cells can also communicate through **gap junctions**, which create *channels* for the intercellular passage of molecules.

Signal transduction usually involves 6 steps:

1. **Synthesis** of the extracellular signal;
2. **Release (secretion)** of the signaling molecule from the signaling cell;
3. **Transport** of the signal to the target cell;
4. **Binding** of the signal by a specific receptor;
5. **Changes in cell metabolism, function, or development initiated by the ligand-receptor complex** - *the main characteristic of hormones*;
6. **Removal** of the signal, resulting in the termination of the cellular response (signal attenuation and receptor desensitization).

structural organization of the signaling pathway (Fig. 3-2)



- **extracellular signal** - binds to receptors;
- **receptor** (membrane or intracellular) - recognizes and interacts with a specific ligand;
- **intracellular signal proteins** - transmit the signal to the target protein;
- **effector molecules** - converting the signal into a biological response.

Fig. 3-2 Structural organization of a signaling pathway

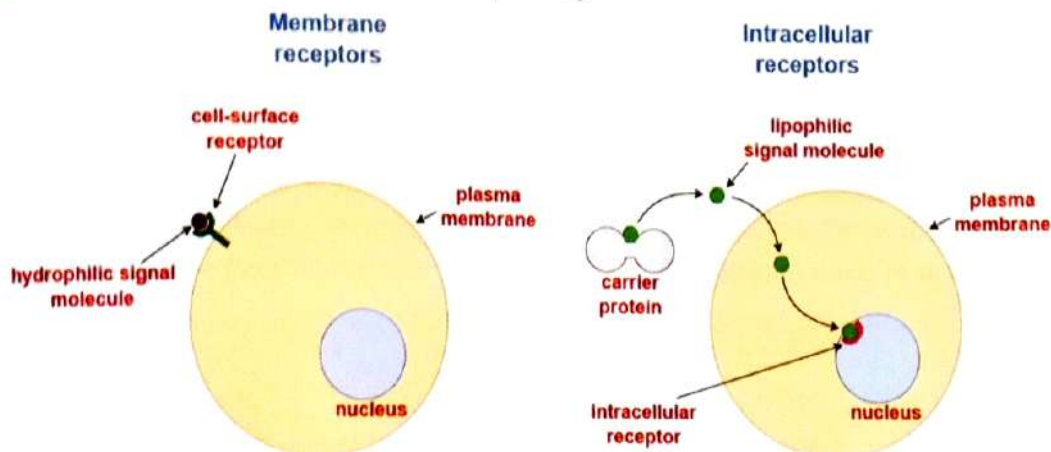


Fig. 3-3 Types of extracellular signals (ligands)

Types of extracellular signals (Fig. 3-3)

1. Water-soluble ligands (hydrophilic, polar)

They **cannot** diffuse through the cell membrane (because they are **water insoluble**, "like dissolves like"). Therefore, they need **membrane receptors**. Examples of such ligands are *amines, amino acids, catecholamines, peptides and proteins e.g.: insulin, glucagon, adrenaline, serotonin, etc.*

2. Lipid-soluble ligands (hydrophobic, lipophilic, non-polar)

They **can** pass freely through the cell membrane and bind to **cytosolic/nuclear receptors (intracellular)**. The ligand-receptor complexes then diffuse across the nuclear membrane and enter the nucleus, where they modulate DNA transcription. Examples include: *steroid hormones, T3/T4, calcitriol and retinoic acid.*

4. Types of plasma membrane receptors

Classification:

- I. Receptors associated with ion channels
- II. G-protein-coupled (guanine nucleotide-binding proteins) receptors
- III. Receptors with enzymatic activity:
 1. Receptors with protein kinase activity:
 - with tyrosine kinase
 - with serine/threonine kinase
 - double-specific
 2. Receptors with protein phosphatase activity
 3. Receptors with guanylate cyclase activity
- IV. Receptors associated with other tyrosine kinases
- V. Death- receptors associated with apoptosis
- VI. Integrins (receptors regulating cell adhesion)

Receptors associated with ion channels

An example of such receptors is the acetylcholine receptor (Fig. 4-1). It is composed of 4 types subunits – α 2, β , γ , and δ . These form an ion channel. The β , γ , and δ subunits undergo **phosphorylation** upon association with an intracellular **protein tyrosine kinase**.

Two classes of acetylcholine receptors have been identified based on their response to the alkaloids muscarine or nicotine – **muscarinic and nicotinic**. Activation of acetylcholine receptors through binding of acetylcholine (see Fig. 5-17 from Medical Biochemistry Part I) leads to *the influx of sodium ions into the cell and efflux of potassium ions*, resulting in **depolarization of postsynaptic neurons** and initiation of a new action potential.

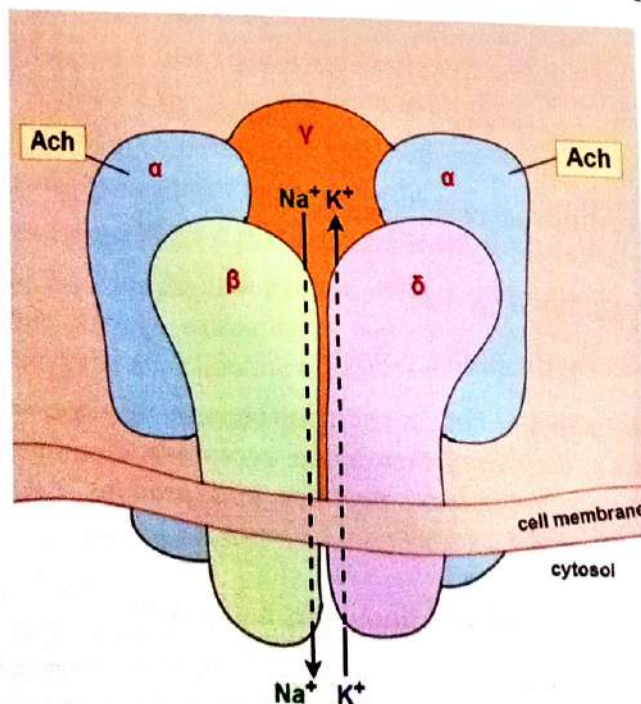


Fig. 4-1 Acetylcholine receptor

G-protein coupled receptors

Each G-protein-coupled receptor has 7 *transmembrane α -helical segments*. The primary messenger (mediator, signaling molecule) binds to the extracellular region of the receptor. This leads to the activation of its intracellular region and subsequently the *G protein* (Fig. 4-2).

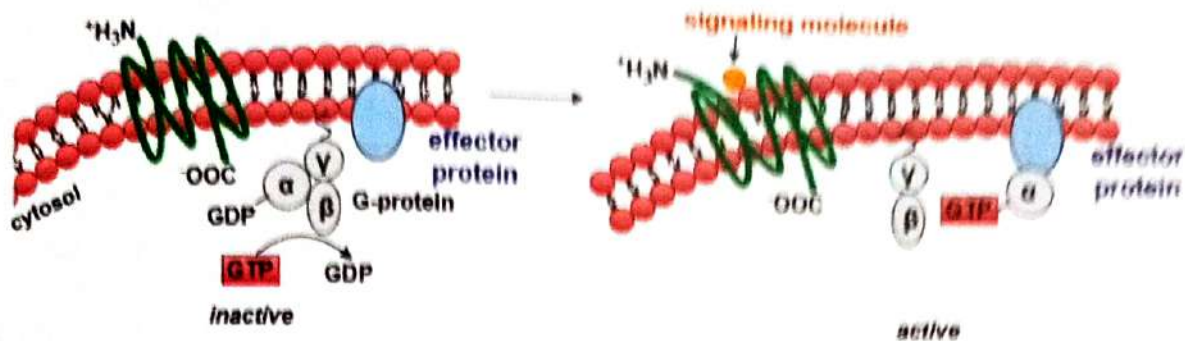


Fig. 4-2 G-protein coupled receptor

General pathway with G-proteins

G-proteins transduce signals from multiple receptors to various targets. The components of such a pathway include:

1. **Membrane receptor** - activated by an extracellular signal.
2. **G-protein** - activated by binding to GTP.
3. **Effector protein** - a target protein activated (or sometimes inhibited) by the G-protein. Sometimes, the effector is a membrane-associated enzyme (e.g., adenylate cyclase).
4. **Second messenger** - a small molecule (e.g., cAMP) released as a result of effector activation.

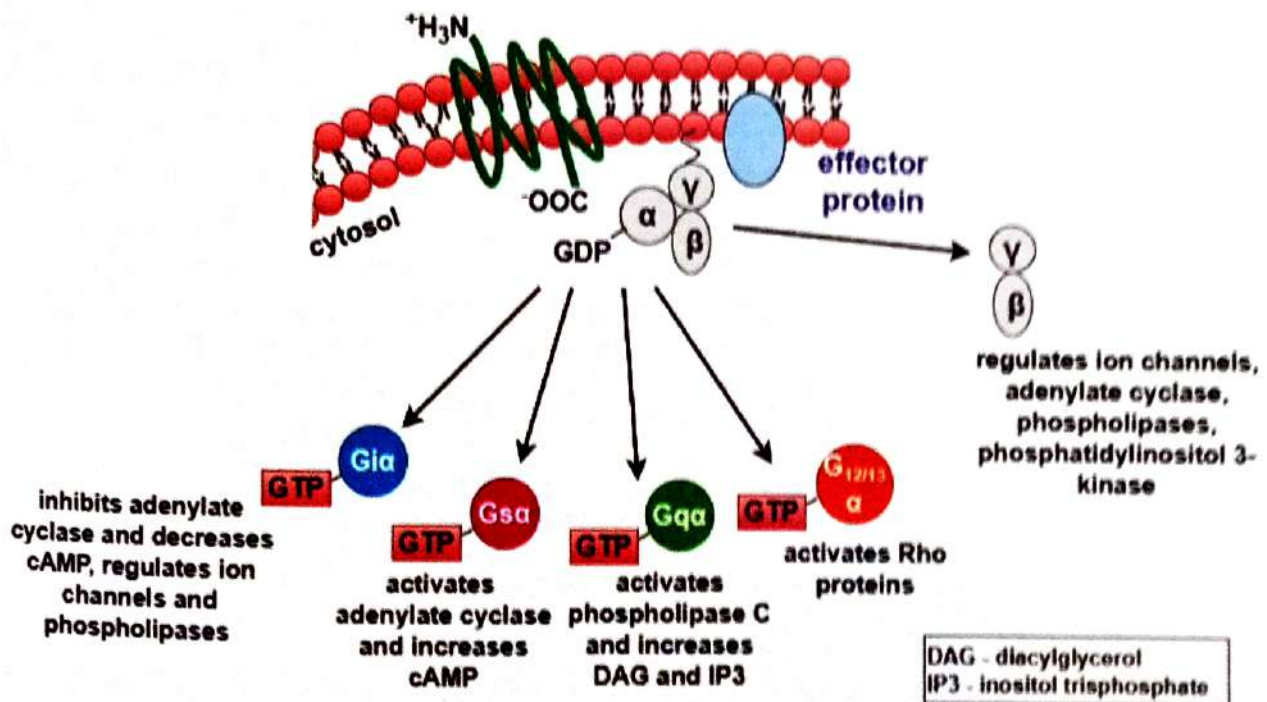


Fig. 4-3 Diversity of biological responses upon activation of G- protein dependent receptors

Heterotrimeric G-proteins (Fig. 4-3)

These regulate signal transduction from cellular receptors to intracellular effectors such as *adenylate cyclase*, *phospholipase C (PLC)*, *cGMP-phosphodiesterase*, *ion channel systems*, *MAPK pathways*, and others.

The G-protein consists of three different subunits: α , β and γ .

- The **α subunit** determines effector specificity and has a *GTP-binding site*.
- The **β and γ subunits** directly regulate effectors like *phospholipase A2*, *phospholipase C- β* , *adenylate cyclase*, and *ion channels*.

There are 4 major α -subunit subfamilies: **Gsa**, **Gia**, **Gqa**, and **G_{12/13} α** .

Gsa - activates *adenylate cyclase* and *Ca²⁺ channels* in all tissues. It is a substrate of cholera toxin.

Gia - activates *PLC* and *PLA2* and *K⁺ channels* and inhibits *adenylate cyclase* and *Ca²⁺ channels*. It is found in almost all tissues. Gia activates the enzyme *cGMP-phosphodiesterase* in the retina, involved in visual reception.

Gqa - activates *PLC*, found in all tissues.

G_{12/13} α - found in all tissues, *regulates Na⁺/H⁺ antiport*, *electrical Ca²⁺ dependent channels*, *eicosanoid cell signals*, *Rho proteins*.

Other members of the G-protein family include G11, G14, G15, and G16, found in hematopoietic tissues.

G-protein subunits undergo post-translational modifications such as *palmitoylation*, *farnesylation*, and *geranylation*. These hydrophobic groups are necessary for proper membrane **anchoring** of the subunits.

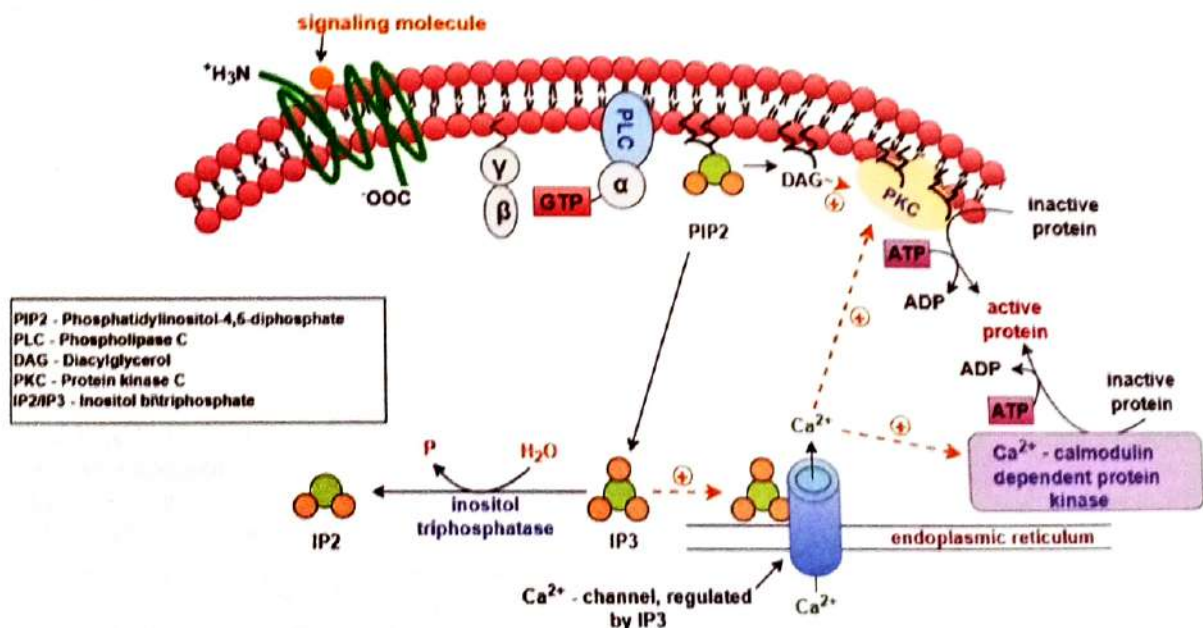


Fig. 4-4 G-protein coupled receptors signaling with lipid mediators

G protein-coupled receptors signaling with lipid mediators (Fig. 4-4)

Certain hormones such as *angiotensin II*, *adrenaline* (via α 1-receptors), *vasopressin*, and *oxytocin* stimulate *phospholipase C* activity in the plasma membrane via **G_q**. PLC hydrolyzes the membrane phospholipid **phosphatidylinositol-4,5-diphosphate (PIP₂)**, producing the lipid mediators **inositol-triphosphate (IP₃)** and **diacylglycerol (DAG)**.

- **IP3** is water-soluble and mobilizes Ca^{2+} from intracellular stores.
- **DAG** remains in the membrane and stimulates protein kinase C.

Ca^{2+} is a potent enzymatic activator, and its cytoplasmic availability is tightly regulated. The response is usually fast and transient, parallel with the rate of muscle contraction.

Clinical aspects of intracellular G-protein signaling

ADP-Ribosylation of G-Proteins

Several bacterial toxins catalyze the covalent attachment of adenosine diphosphate (ADP)-ribose to G-proteins:

- **Cholera toxin** performs ADP-ribosylation of the *G α* subunit. This results in its *constant activation* and an *inability* to hydrolyze GTP. This primarily affects the intestinal mucosa, causing *excessive secretion of water and electrolytes* (i.e., diarrhea).
- **Pertussis toxin** (*Bordetella pertussis*) performs ADP-ribosylation of the *G $\beta\gamma$* subunit. This results in its *constant inactivation*, preventing the inhibition of adenylate cyclase. This leads to *whooping cough*.
- **Diphtheria toxin** performs ADP-ribosylation of *EF-2* (elongation factor 2). This *blocks* polypeptide synthesis.

Receptors with enzymatic activity

1. Receptors with protein kinase activity

According to their **localization**, there are two groups of protein kinases:

1. Receptor kinases in the **membrane**.
2. **Free** soluble protein kinases in the **cytosol**.

1.1 Receptors with tyrosine kinase activity

Ligands for receptor tyrosine kinases (RTKs) are **growth factors and insulin**.

The primary activity of protein kinases is to **phosphorylate** target proteins through **ATP hydrolysis**.

Receptors have a characteristic structure—they are integral membrane proteins that cross the membrane **once** and have a cytosolic domain. The **N-terminus** is oriented extracellularly, while the **C-terminus** faces the cytosol.

Some receptors, such as those for **EGF and PDGF** (Platelet-derived growth factor), consist of **single polypeptide chains**, whereas the **insulin receptor** is a **heterodimer** ($\alpha 2\beta 2$) with monomers connected by disulfide bonds.

Receptors in this group share a common mechanism of action. Their cytosolic domains exhibit **tyrosine kinase enzymatic activity**.

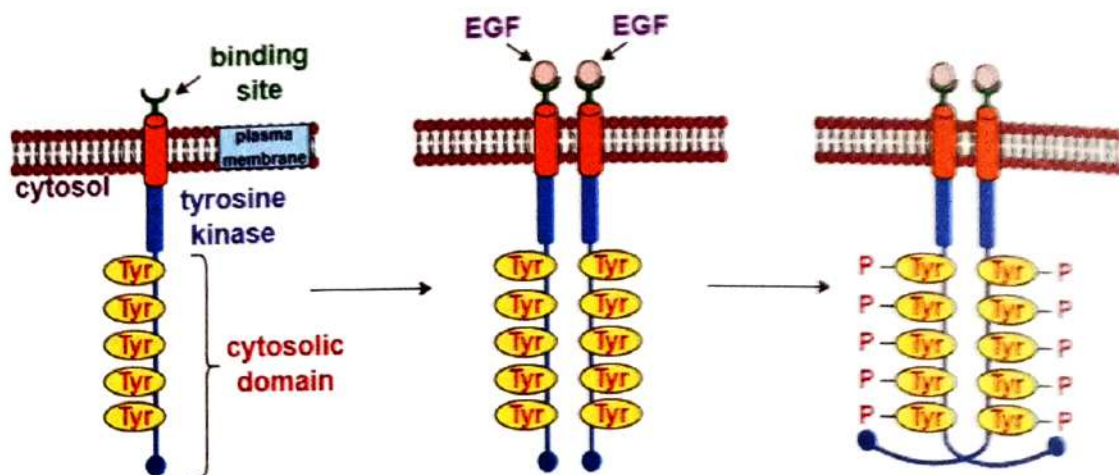


Fig. 4-5 Binding of epidermal growth factor (EGF) to its receptor

Major steps in RTK-mediated signaling pathways (Fig. 4-5)

1. Upon ligand binding, the receptor **dimerizes or oligomerizes**.
2. This is followed by **autophosphorylation** of various tyrosine residues within the cytoplasmic region of the receptor, mediated by its intrinsic tyrosine kinase activity.

Each phosphorylated tyrosine residue outside the kinase domain binds **specific signaling molecules** (e.g., GRB2 – growth factor receptor-bound protein 2, phosphatidylinositol-3-kinase, or phospholipase C), which possess **SH2 domains** (Src homology 2 domain). This leads to the activation of downstream signaling pathways. Autophosphorylated tyrosine residues serve as critical docking sites for these SH2 domain-containing molecules.

Effector pathways of RTKs

RTKs activate two main types of effector pathways:

1. **Enzymatic activity stimulation** leading to the generation of small molecules (**second messengers**), e.g., PLC pathway.
2. **Cascade pathways**, involving sequential interactions between macromolecular components, e.g., MAPK pathway.

Signaling cascades involving MAPK (Mitogen-Activated Protein Kinase)

Several MAPK cascades have been identified in human cells, including **ERK1/2**, **ERK5** (extracellular signal-regulated kinases), and stress-activated kinase pathways such as **JNK/SAPK** and **p38 MAPK** (Jun amino-terminal kinases and stress-activated protein kinase). These pathways have shared components, where one element in one pathway can activate another element in a different pathway.

Both G-protein-coupled receptors and receptor tyrosine kinases participate in these pathways. Most **ligands** include **stress stimuli**, **mitogens**, **cytokines**, **growth factors**, **hormones**, and **neurotransmitters**, which selectively activate these cascades. All MAPK pathways operate through sequential **phosphorylation** processes, culminating in the phosphorylation of transcription factors and the **regulation of gene expression**.



Mitogen-activated protein kinases (MAPKs) are a family of *serine/threonine protein kinases* involved in various cellular programs, including *cell proliferation, differentiation, movement, and cell death*.

Pathway description (Fig. 4-6):

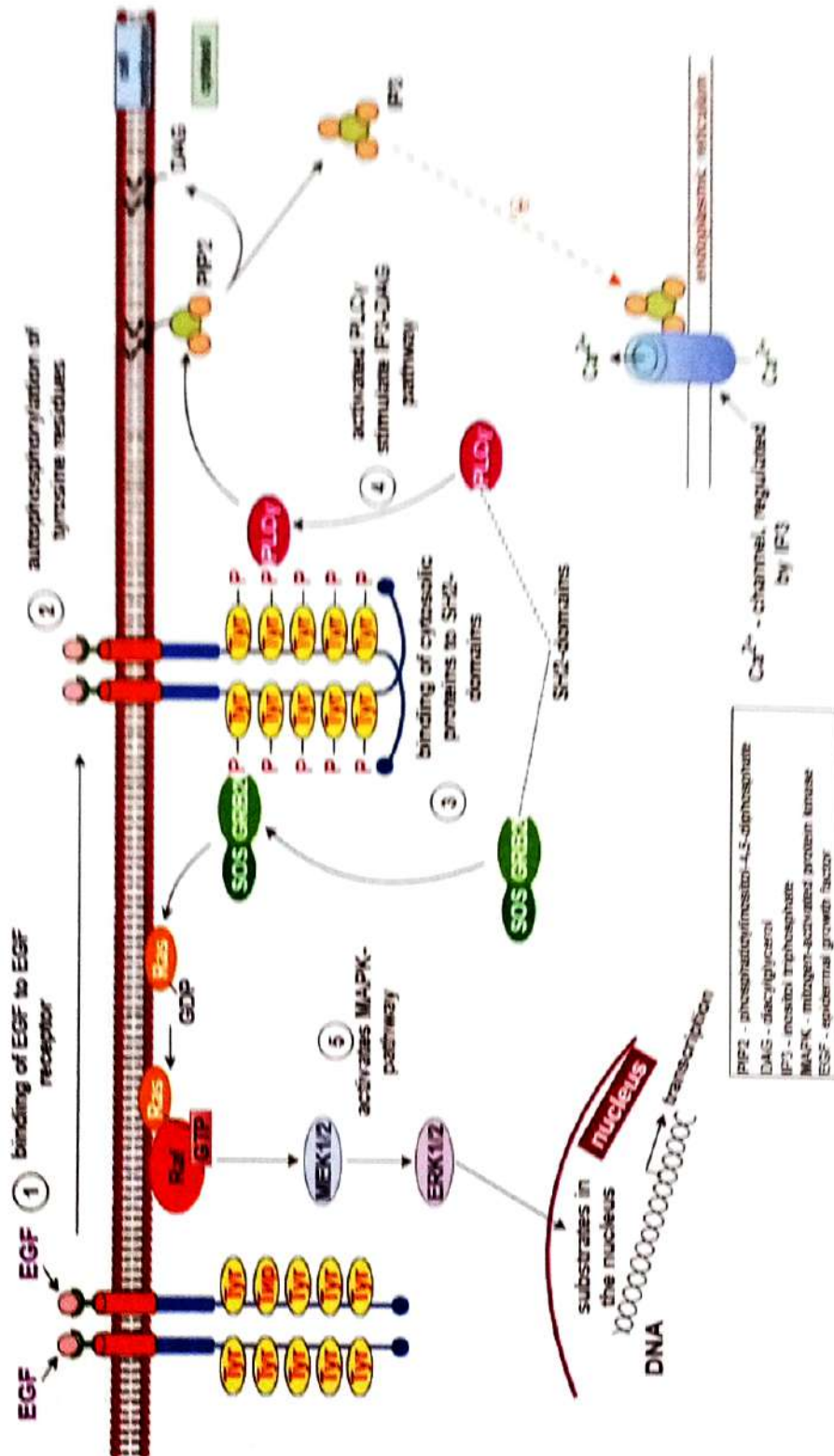
MAPK signaling cascades are hierarchically organized into three interconnected modules.

MAPK (1K) is phosphorylated and activated by MAPK kinases (*MAPKK or 2K*), which in turn are phosphorylated and activated by MAPKK kinases (*MAPKKK or 3K*). MAPKKKs are activated through interactions with proteins from the "small" GTPase family and/or other protein kinases that link MAPK to membrane receptors or external stimuli.

Fig. 4-6 MARK signaling pathways

Signal transduction by receptor tyrosine kinases (Fig. 4-7)

The epidermal growth factor (EGF) utilizes the formation of secondary lipid mediators via *phospholipase C* as an effector pathway, as well as a signaling cascade involving *mitogen-activated protein kinases (MAPKs)*. Ligand binding leads to the activation of the receptor's cytosolic kinase domain, which results in the *autophosphorylation* of tyrosine residues within the receptor. These phosphorylated residues serve as recognition sites for *SH2 domains* of cytosolic proteins—*GRB2* and *PLC*—which initiate the MAPK pathway and the lipid mediator signaling pathway.



ERK1/2 (Fig. 4-7)

One of the MAPK families is called ERK (extracellular signal-related kinase), as it regulates extracellular signals. This pathway involves **Ras**, a "small" *G-protein with GTPase activity*. This signaling pathway is typically activated by a *tyrosine kinase receptor*, such as EGFR and PDGFR.

The receptor activates the *Ras pathway* through an adaptor protein, **GRB2**, which binds to the activated receptor via its *SH2 domain*.

The signal is transmitted by the activation of SOS (son of sevenless). Activation occurs through the binding of the *SH3 domain* of GRB2 to SOS. SOS, another "small" G-protein, displaces GDP from its complex with Ras, allowing Ras to bind GTP and become activated. Activation of Ras leads to the activation of Raf. Raf is a *serine-threonine kinase (MAPKKK)*, which, in turn, activates MEK (MAPK kinase or MAPKK). The name MEK reflects its role as a kinase that phosphorylates and thus activates a MAP kinase (or MAPK).

MEK is known as a *dual-specificity kinase* because it can phosphorylate both *serine/threonine and tyrosine residues*. The target of MEK is *ERK1/2 (or MAPK)*, after which the signal is transmitted to the **nucleus**, where various transcription factors are phosphorylated. Each kinase in this part of the pathway phosphorylates its target kinase, and the phosphorylation event results in an *increase in the activity of the phosphorylated molecule*.

Small G-proteins Molecular switches

Small G-proteins are monomeric with a molecular weight of 20–40 kDa. Similar to heterotrimeric G-proteins, **their activity depends on GTP binding**. More than 100 small G-proteins have been identified, including **Ras, Rho, Rab, Ran, Arf**, and others.

Similar to other G-proteins, cycles of switching the Ras protein into an active and inactive state have been established (Fig. 4-8). Ras can "switch" between a *GTP-bound* and a *GDP-bound* state. The transition from *GDP-bound* to *GTP-bound* state is catalyzed by **GEF (guanine nucleotide exchange factor)**,

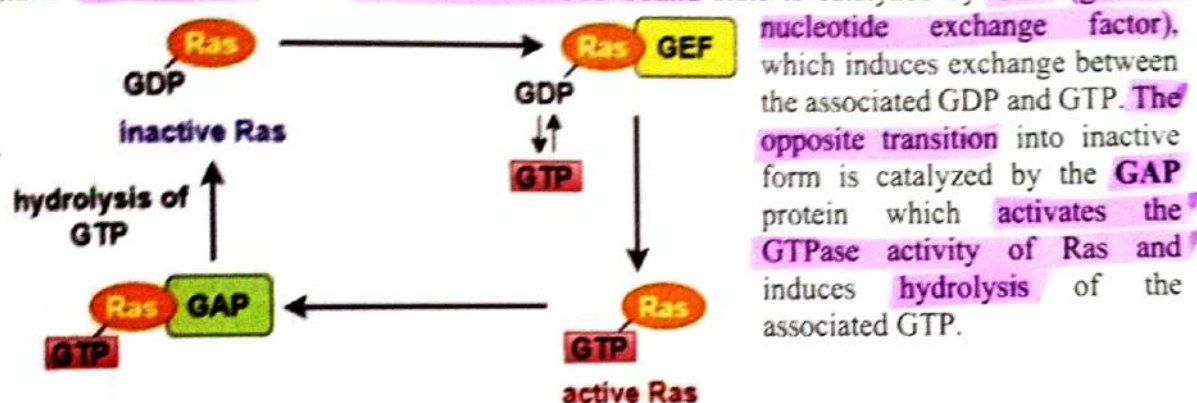


Fig. 4-8 Cycles of Ras

Ras and cancer (see Fig. 12-1)

Mammalian cells contain 3 types of Ras: H, K, and N.

Mutations of Ras proto-oncogenes (**H-Ras, N-Ras, K-Ras**) are found in about 25% of all human tumors. Most of these mutations result in the **elimination of the normal GTPase activity of Ras**. This prevents the hydrolysis of GTP to GDP, thus "locking" the Ras protein in an active state, leading to **uncontrolled activation of downstream signaling pathways**. Mutated forms of Ras can strongly bind GAP (the GTPase-activating protein) but are unable to catalyze the hydrolysis of GTP.

40% of patients with colorectal carcinoma have mutations in the K-Ras gene.

Malignant transformation can be enhanced due to the *overstimulation* of Ras signaling pathways, which either *stimulate cell growth or inhibit apoptosis*.

p38 and JNK pathways

The **JNK (c-Jun amino-terminal kinase)** pathway and **p38 MAP kinase** are activated in response to stress stimuli, e.g., UV rays. JNK and other MAPKs (excluding MEK) phosphorylate serine or threonine residues. Various "small" G-proteins are involved in

MAPK pathways. E.g., the ERK pathway involves **Ras**, which is activated by PI3K (phosphatidylinositol-3-kinase). Another small G-protein is **Rho**, which initiates the formation of actin "stress" filaments at their contact points with the plasma membrane, called **focal adhesions** (see Fig. 15-8). Rho can be activated by growth factors.

1.2 Receptors with serine/threonine kinase activity

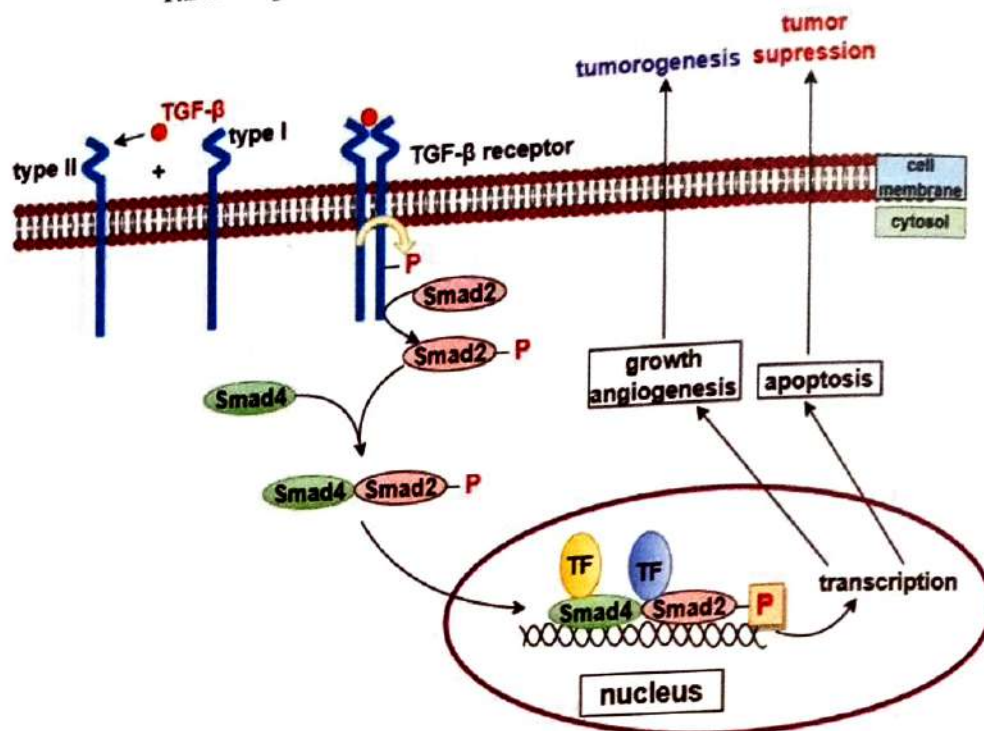


Fig. 4-9 TGF- β signaling pathway

In addition to **JNK** and most **MAPKs**, the receptors from *the transforming growth factor-beta (TGF- β) family* also function in a similar manner (Fig. 4-9). The TGF- β receptor consists of two types of subunits, both of which possess *serine/threonine kinase activity*. The signaling pathway follows this sequence:

1. The ligand binds to the **type II** receptor, creating a ligand-receptor complex with high affinity for the **type I** receptor.
2. A complex is formed between the two types of receptors, within which the type II receptor phosphorylates the type I receptor.
3. When activated, the type I receptor phosphorylates a transcription factor of the **Smad** family.
4. The phosphorylated **Smad 2** then dissociates from the receptor and forms a complex with **Smad 4**.
5. The heterodimer then moves into the nucleus where it binds to DNA and activates transcription.

2. Receptors with protein phosphatase activity

Transmembrane protein tyrosine phosphatases (PTPs)

The first transmembrane protein tyrosine phosphatase (PTP) characterized was the **leukocyte common antigen protein, CD45**. This protein is homologous to the intracellular protein tyrosine phosphatase PTP1B. CD45 is involved in regulating the *tyrosine kinase activity* of the *lymphocyte-specific protein tyrosine kinase (Lck)* in **T cells**. Lck is a tyrosine kinase from the

Src kinase family. Lck is associated with the T cell antigens CD4 and CD8, forming a **complex** that participates in T cell activation. CD45 dephosphorylates the C-terminus of Lck and activates it.

3. Receptors with guanylate cyclase activity

Receptors for natriuretic hormones with guanylate cyclase activity

Three different receptors for natriuretic hormones, also called *atrial natriuretic peptides (ANP)*, have been identified: **ANPR-A**, **ANPR-B**, and **ANPR-C**. Binding of these peptides to their receptors activates **guanylate cyclase (GC)**, leading to the formation of **cGMP**. Both **ANPR-A** and **ANPR-B** receptors cross the plasma membrane, and their intracellular domains possess intrinsic *guanylate cyclase activity*. **ANPR-C** does not contain an intracellular domain with guanylate cyclase activity. It acts through a G-protein that **activates PLC- γ** and inhibits **adenylate cyclase** or simply acts as a receptor removing natriuretic peptides from the blood. Activation of GC by the receptor leads to the conversion of **GTP to cGMP**.

Nitric oxide (NO) also stimulates cGMP production by activating **soluble GC** (see Fig. 5-2). Like cAMP, cGMP mediates most of its intracellular effects through the activation of a specific cGMP-dependent **protein kinase G (PKG)**.

Several families of **phosphodiesterases (PDE-I-VI)** act as *regulatory switches* catalyzing the degradation of cGMP to guanosine-5'-monophosphate (5'-GMP).

The short (10-second) lifetime of NO limits its action close to its source of synthesis. However, it easily crosses membranes to enter target cells. Nitric oxide also stimulates bactericidal activity in macrophages, inhibits platelet aggregation, and serves as a neurotransmitter in the brain.

Clinical aspects of intracellular cGMP signaling

Erectile Dysfunction

The mechanism of penile erection involves the release of NO into the *corpus cavernosum* as a result of sexual stimulation. NO activates the enzyme **guanylate cyclase**, leading to **increased levels of cGMP**, which results in **smooth muscle relaxation** in the corpus cavernosum and allows blood flow. Drugs for the treatment of erectile dysfunction enhance the effect of NO by **inhibiting phosphodiesterase type 5 (PDE5)**, which is responsible for the breakdown of cGMP in corpus cavernosum. The results are smooth muscle relaxation and an increased blood flow to the corpus cavernosum.

Receptors associated with the functions of other tyrosine kinases

Receptors for many cytokines and growth factors lack intrinsic protein kinase activity but are associated on their intracellular side with other **tyrosine kinases** or **serine/threonine kinases**. One such tyrosine kinase is called **Jak (Janus kinase, just another kinase)**, which can activate downstream effectors that include the STAT proteins - **Jak/STAT pathway** (Fig. 4-10).

Jak has seven homologous regions called *Janus homology domains* 1 to 7 (JH1-7). JH1 is a kinase domain and JH2 is a negative regulator of JH1 activity. It is named after the two-faced **God Janus** symbolizing its dual regulatory functions.

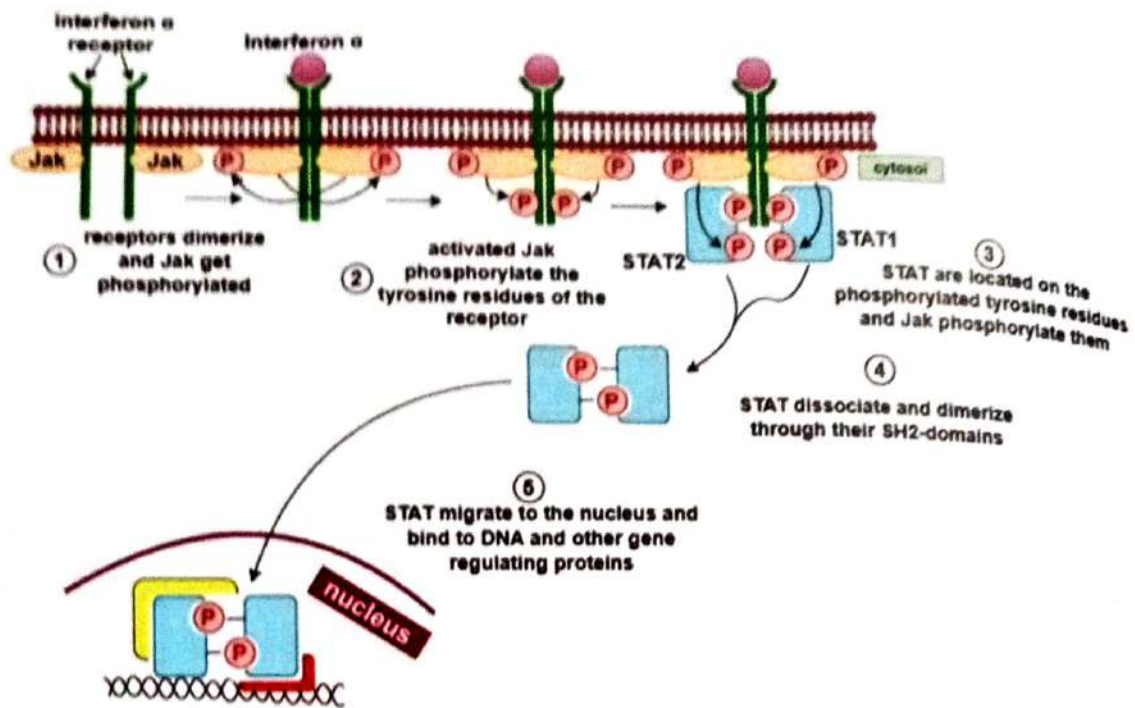


Fig. 4-10 Jak/STAT signaling pathway



5. Participation of secondary mediators in signal transduction

The extracellular signal is referred to as a *primary messenger*. Signal transmission inside the cell is mediated by *secondary messengers*, which include:

1. **Cyclic nucleotides:**
 - **cAMP** - activates protein kinase A (PKA - cAMP-dependent protein kinase A);
 - **cGMP** - activates protein kinase G (PKG).
2. **Lipid mediators:**
 - **Diacylglycerol (DAG)** - activates protein kinase C (PKC);
 - **Inositol triphosphate (IP₃)** - increases intracellular Ca²⁺ levels and also activates PKC;
 - **Ceramide;**
 - **Sphingosine-1-phosphate.**
3. **Ca²⁺ and calmodulin** - activate several protein kinases known as Ca²⁺/calmodulin-dependent protein kinases I, II, and III.
4. **Hormone-receptor complex** (for lipophilic hormones).

cAMP in signal transduction

cAMP

The primary source of cAMP is ATP (adenosine triphosphate).

Cyclic adenosine 3'5'-monophosphate (cAMP) is the first documented secondary messenger, playing a key role in cellular responses to many extracellular stimuli. Various receptors, mostly *GPCRs* (*G-protein coupled receptors*), *α- and β-adrenergic receptors*, *growth factor receptors*, *hypothalamic CRHR* (*Corticotropin Releasing Hormone Receptor*), *GcgR* (*Glucagon Receptor*), and others, are responsible for the accumulation of cAMP in cells, leading to various physiological outcomes. Changes in cAMP levels selectively activate **PKA isoforms**. The main source of cAMP is ATP.

Adenylate cyclase (AC)

The conversion of ATP to cAMP is carried out by members of the class III adenylate cyclase (EO 4.6.1.1), which in humans consists of nine transmembrane AC enzymes and one soluble AC.

1. **Transmembrane ACs** are regulated by different types of **G-protein coupled receptors** and therefore have a key role in the cellular response to extracellular signals.
2. **Soluble AC**, on the other hand, is insensitive to G-proteins, but is *directly activated by Ca²⁺ (calcium ions) and the metabolite HCO₃⁻ (bicarbonate ion)*, which makes the enzyme an intracellular metabolic sensor.

Regulation of adenylate cyclases by G-proteins - see fig. 4-3

Extracellular stimuli such as *neurotransmitters*, *hormones*, *inflammatory stimuli*, *stress*, *adrenaline*, *noradrenaline*, etc. activate G-proteins through *G-protein coupled receptors*.

The **adenylate cyclase system** is regulated by at least two types of G-proteins - G_s (stimulatory) and G_i (inhibitory).

In the absence of a hormone, the heterotrimeric G-complex is in an *inactive GDP-bound form* and is not associated with the receptor. This complex is anchored to the plasma membrane by *prenylated* groups on the $\beta\gamma$ -subunits and by *myristoylated* groups on the α -subunits. *When the hormone binds to the receptor*, a **conformational change** occurs in the receptor, activating the **G-protein complex**. This leads to **replacement** of GDP with GTP on the α -subunit, after which the α - and $\beta\gamma$ -subunits **dissociate** (separate). The α -subunit binds to and modulate the **effector**, which can be *adenylate cyclase*, *phospholipase C*, K^+ - channel or other molecules.

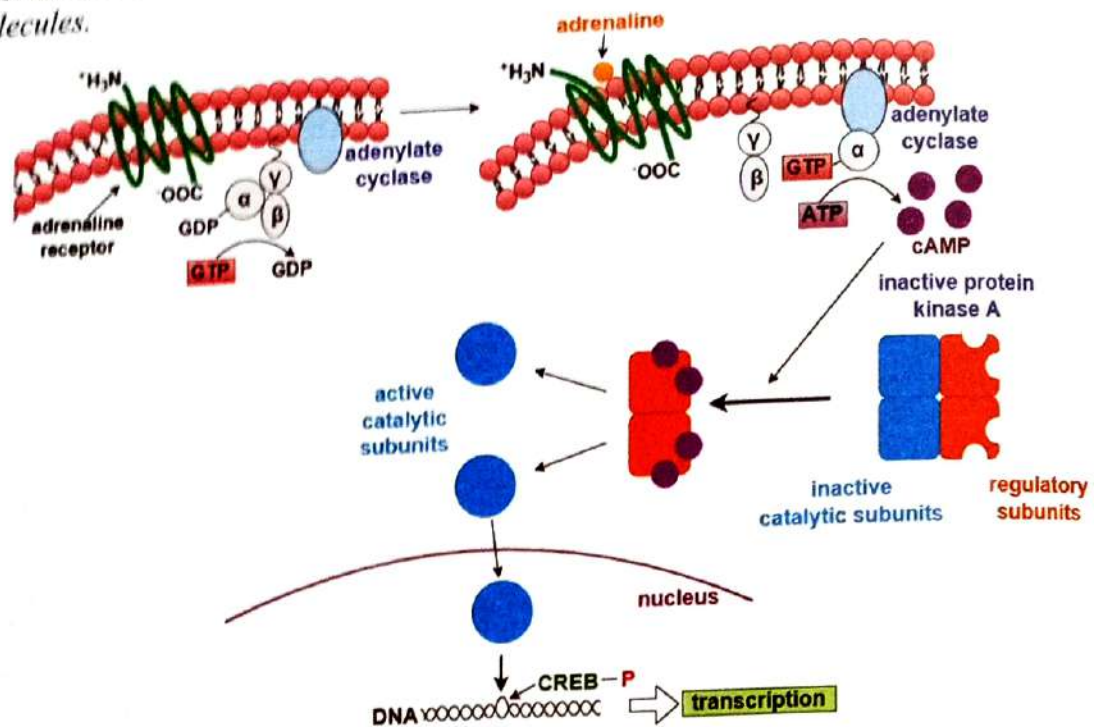


Fig. 5-1 Activation of adenylyl cyclase system

Activation of the adenylyl cyclase system by adrenaline (Fig. 5-1)

This system consists of a membrane-located adrenergic **receptor**, a **G-protein**, and the enzyme **adenylate cyclase**. **Protein kinase A (PKA)** is activated.

In the inactive state, the α -subunit of the G-protein contains GDP, whereas in its active state, it binds GTP. cAMP binds to PKA, which is a tetrameric molecule consisting of 2 regulatory and 2 catalytic subunits. Binding of cAMP to the regulatory subunits, which are membrane-associated, releases the two catalytic subunits in their active form. These catalytic subunits can phosphorylate various substrates and translocate to the nucleus.

cAMP is related to control of transcription by activation of transcription factor **CREB** (*cAMP response element binding protein*). CREB is one of the main substrates for PKA. Phosphorylation of a serine residue strongly increases the activity of CREB, allowing it to bind to a region of DNA known as **CRE** (*cAMP response element*), which is found in genes whose transcription is regulated by cAMP.

The expression of genes encoding proteins involved in catabolic pathways is activated (e.g., glycolysis, glycogenolysis, lipolysis, etc., see Fig. 1-36, Fig. 4-11 from Medical biochemistry Part I).

Nitric oxide and cGMP in signal transduction

NO (nitric oxide)

Nitric oxide (NO) has a **short half-life**. It is both a gas and a free radical and is involved in various physiological and pathological processes. NO is formed from **L-arginine** simultaneously with L-citrulline, catalyzed by three different isoforms of **nitric oxide synthase** - NOS (NO synthases) (see Fig. 5-16 from Medical biochemistry Part I).

Mechanism of action of NO:

1. Activation of soluble Guanylate Cyclase (sGC):

- NO diffuses freely across cell membranes into target cells.
- In the cytoplasm, NO binds to the heme moiety of **soluble guanylate cyclase (sGC)**, activating the enzyme.
- Activated sGC converts **GTP (guanosine triphosphate)** to **cyclic GMP (cGMP)**.
- cGMP acts as a second messenger to mediate various effects, such as *smooth muscle relaxation, vasodilation, and platelet inhibition*.

2. Post-translational modifications:

- NO modifies proteins through **S-nitrosylation**, which involves the addition of an NO group to *cysteine residues* in proteins.
- This modification alters protein activity, stability, or localization, influencing cellular functions like *signal transduction and apoptosis*.

3. Regulation of mitochondrial function:

- NO interacts with mitochondrial *cytochrome c oxidase*, temporarily inhibiting respiration and reducing reactive oxygen species (ROS) production.
- *At high concentrations*, NO can lead to mitochondrial dysfunction and apoptosis.

4. Anti-inflammatory effects:

- NO *reduces* the expression of pro-inflammatory cytokines and adhesion molecules on endothelial cells, limiting leukocyte recruitment and inflammation.

Type - I nNOS (neuronal NOS) and type-III eNOS (endothelial NOS) are expressed **constitutively (permanently)** as latent enzymes and require a higher concentration of Ca^{2+} for manifestation of enzyme activity.

In contrast, **type-II iNOS (inducible NOS)** is Ca^{2+} independent, due to its high affinity for $\text{Ca}^{2+}/\text{CaM}$ (calmodulin), making the enzyme active even at basal levels of intracellular Ca^{2+} levels.

NO diffuses into neighboring cells where it stimulates **soluble guanylate cyclase** to form **cGMP**, which regulates several enzymes and ion channels.

eNOS

NO is produced by **endothelial cells** in response to **mechanical forces** such as e.g. **shear stress** and **humoral factors** such as *growth factors, acetylcholine, VEGF (vascular endothelial growth factor), BDK (bradykinin), estrogens, S-1P (sphingosine 1 - phosphate), H_2O_2 (hydrogen peroxide) and angiotensin-II*.

1. Myristoylation and Palmitoylation direct eNOS to caveolae

- **Myristoylation** – a lipid modification that anchors eNOS to membranes.

- *Palmitoylation* – further stabilizes eNOS at caveolae, specialized microdomains in the endothelial plasma membrane rich in caveolin-1.

2. Caveolin-1 inhibits eNOS

- Caveolin-1 binds to and inhibits eNOS activity in its inactive state.

3. Calmodulin (CaM) relieves caveolin inhibition

- When intracellular Ca^{2+} levels rise, CaM binds to eNOS.
- This displaces eNOS from caveolin-1, activating NO production.

4. hsp90 enhances eNOS activation

- hsp90 (Heat Shock Protein 90) interacts with eNOS and stabilizes its active conformation.
- This helps calmodulin to efficiently displace caveolin-1, further enhancing NO production.

5. Akt (Protein Kinase B) phosphorylation increases eNOS activity

- Akt phosphorylates eNOS at Ser1177, leading to increased enzyme activity, even at lower Ca^{2+} levels.

Biological significance of eNOS - synthesizes NO in the endothelium, which:

1. Acts as a *vasodilator* and lowers blood pressure.
2. *Inhibits platelet aggregation, and the adhesion* of platelets and leukocytes to the endothelium. It also affects endothelin-1 production, vascular smooth muscle cell proliferation, and angiogenesis.

Due to NO's critical role in these processes, it is believed that disturbances in endothelial NO production contribute to the pathogenesis of certain vascular disorders such as **atherosclerosis and hypertension**.

NO plays a role in the *relaxation of smooth muscle cells* in the vascular wall, facilitated by acetylcholine, which is released from nerve terminals in blood vessels. This activates eNOS in the endothelium (Fig. 5-2). The mechanism by which acetylcholine leads to smooth muscle relaxation explains the action mechanism of the drug **nitroglycerin**.

After inducing relaxation, cGMP is rapidly converted into GMP by **phosphodiesterase**. The well-known drug for impotence, **Viagra** (sildenafil citrate), *inhibits phosphodiesterase 5*.

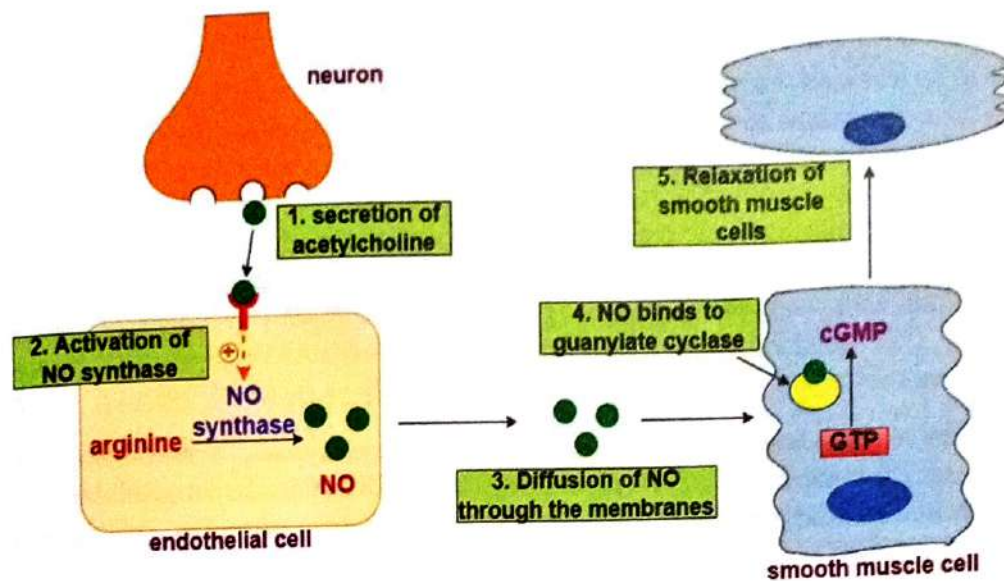


Fig. 5-2 Role of NO in the relaxation of smooth muscle cells in the vascular wall

iNOS

Inducible NO synthase (iNOS) is produced in **macrophages**. After activation by **endotoxins** or **cytokines**, an abundant amount of NO is generated, which has bactericidal activity against invading microorganisms. NO and superoxide anion are major effector molecules in the nonspecific immune system, directly inhibiting pathogen replication. Various extracellular stimuli can activate signaling pathways that converge and initiate iNOS expression:

1. **Bacterial and fungal cell wall** components trigger a cascade of non-specific immune defense, that leads to the expression of iNOS.
2. **Cytokines** released by the infected cells of the affected organism also activate the formation of NO.

Ca²⁺ and calmodulin

Ca²⁺ signaling pathways

Ca²⁺ can activate two different signaling pathways:

1. In **nerve, muscle, and endocrine pancreatic cells**, which are electrically active (excitable) cells, **depolarization** of the plasma membrane **causes the influx of Ca²⁺ from the extracellular space**, initiating the biological response (e.g. secretion of a neurotransmitter or hormone). Ca²⁺ enters through electrical channels (**Voltage-dependent Ca²⁺ channels - VDCC**), which open when the plasma membrane is depolarized (i.e., through an *action potential* in nerve and muscle cells **or** by an increase in intracellular ATP (from glucose metabolism) which closes *K⁺-ATP channels*, leading to membrane depolarization and Ca²⁺ influx in endocrine pancreatic-β cells).

2. In **electrically non-excitable cells**, the binding of extracellular signaling molecules to cell surface membrane receptors induces the entry of Ca²⁺ through **voltage-independent Ca²⁺ channels (VICC)** or the release of Ca²⁺ from the **endoplasmic reticulum (ER)**. In the latter case, events at the cell surface are coupled with the opening of Ca²⁺ channels in the ER via **inositol triphosphate (IP₃)**.

Defects in genes encoding the proteins of Ca²⁺ channels, Ca²⁺-ATPases, and intracellular Ca²⁺ sensors are associated with diseases or mortality.

Ca²⁺ as a secondary mediator

Ca²⁺ acts as an intracellular (secondary) mediator in many cellular responses.

1. **Short-term responses** to Ca²⁺ involve participation in **excitation-contraction coupling** in **skeletal and cardiac muscle** and participation in **stimulus-secretion processes** in **endocrine and nerve cells**.

The mechanisms involved in these processes include, in particular, post-translational modifications of ion channel subunits, e.g., by **phosphorylation** catalyzed by **Ca²⁺ - activated kinases** (*calmodulin - dependent kinase II*, *Ca²⁺/phospholipid-dependent protein kinase*). Additionally, **Ca²⁺-sensitive adenylate cyclases** can **increase cAMP levels**, further **enhancing protein phosphorylation** via *protein kinase A (PKA)*.

2. **Long-term responses** to Ca²⁺ include gene expression, cellular proliferation, differentiation, apoptosis, etc. Proteins whose **expression** is regulated by changes in intracellular Ca²⁺ levels include **hormones, neuropeptides, ion channels, and oncogene proteins**. Decreased entry of Ca²⁺ into the cytosol **inhibits** cell proliferation.

Calmodulin (CaM)

Calmodulin is a cytosolic calcium-dependent protein that is homologous in structure and function with the muscle protein **troponin C**. CaM does not have an enzymatic function itself—it acts as an **allosteric regulator** by binding to and activating other proteins. **CaM** often functions as a subunit in more complex protein assemblies and is involved in the **regulation of various kinases and enzymes** from the metabolism of cyclic nucleotides, as well as in the control of ion transport. **Ca²⁺/CaM** regulates the activity of many structural elements of the cell. They include the **smooth muscle contraction** and various microfilament-mediated processes, including **cellular movements, mitosis, granule release and endocytosis**.

Lipid mediators

1. **Lipid mediators formed in the signaling pathway of phospholipase A2** - see fig. 4-21 from Medical Biochemistry Part I

A lot of the lipids involved as **second messengers** in cell signaling pathways are formed in the **arachidonic acid (AA) pathway**.

AA is an unsaturated fatty acid that is normally found as a constituent of membrane phospholipids and is released from them upon hydrolysis by **phospholipase A2 (PLA2)**.

Prostaglandins (PG) are formed by the cyclooxygenase pathway, in which AA is degraded by the enzyme **cyclooxygenase (COX)**. There are both a **constitutive (COX-1)** and an **inducible form of cyclooxygenase (COX-2)**. Additionally, prostacyclins (**PGI₂**) and **thromboxanes (TXA)** are also formed.

Another group of compounds in this class are **leukotrienes (LT)** and **lipoxins (LX)**. Leukotrienes are formed directly from AA by **5-lipoxygenase**, forming **5-hydroperoxyeicosatetraenoic acid (5-HPETE)**, subsequently converted into **LTA₄**.

LTA₄ is an epoxide intermediate, rapidly converted into **either LTB₄**, which **induces inflammation** through its chemotactic and degranulating effects on polymorphonuclear leukocytes, and also of **LTC₄, LTD₄, and LTE₄** (cysteinyl-containing leukotrienes) that induce vasoconstriction and bronchospasm and are also involved in the pathogenesis of **asthma and anaphylaxis**.

2. Lipid mediators formed in the phosphatidylinositol pathway

Phospholipase C pathway

Phosphatidylinositol-4,5-bisphosphate (PIP₂) is a phosphorylated glycerophospholipid. It is a minor but functionally significant component of the inner layer of the plasma membrane. As a result of the hydrolytic activity of membrane phospholipase C, two important lipid mediators are cleaved from PIP₂: **inositol-1,4,5-triphosphate (IP₃)** and **diacylglycerol (DAG)**. IP₃ mobilizes Ca²⁺ from intracellular stores, and DAG stimulates protein kinase C (see Fig. 4-4).

Phosphatidylinositol-3-kinase (PI3K) pathway (Fig. 5-3)

PI3K is a heterodimeric protein (enzyme) containing one 85 kDa and one 110 kDa subunits. PI3K is activated by **receptors with tyrosine kinase activity** for **insulin** and **various growth factors** (*PDGF* (Platelet-Derived Growth Factor), *EGF* (Epidermal Growth Factor), *IGF-1* (Insulin-Like Growth Factor 1), *HGF* (Hepatocyte Growth Factor), *NGF* (Nerve Growth Factor)).

PI3K phosphorylates inositol at the 3rd position, converting PIP₂ to **phosphatidylinositol-3,4,5-triphosphate (PIP₃)**, which is a key second messenger.

PIP₃ attracts **PDK1** (phosphoinositide-dependent protein kinase) to the plasma membrane and activates it by binding to the PH domain (Pleckstrin homology) of **Akt** (also called protein kinase B). **PDK1** phosphorylates and activates **Akt**, which regulates various cellular processes. **Akt** needs a second phosphorylation event (by **mTORC2**) for full activation. **PTEN** (phosphatase and tensin homolog) is a *tumor suppressor* that dephosphorylates PIP₃, converting it back to PIP₂. This reduces **Akt** activation and thus suppresses cell survival and growth signals. PI3K is involved in the regulation of numerous cellular functions such as *cell adhesion*, *chemotaxis*, *apoptosis*, *secretory responses*, *insulin-mediated membrane translocation of glucose transporters*, *platelet activation*, and *cytoskeletal reorganization*.

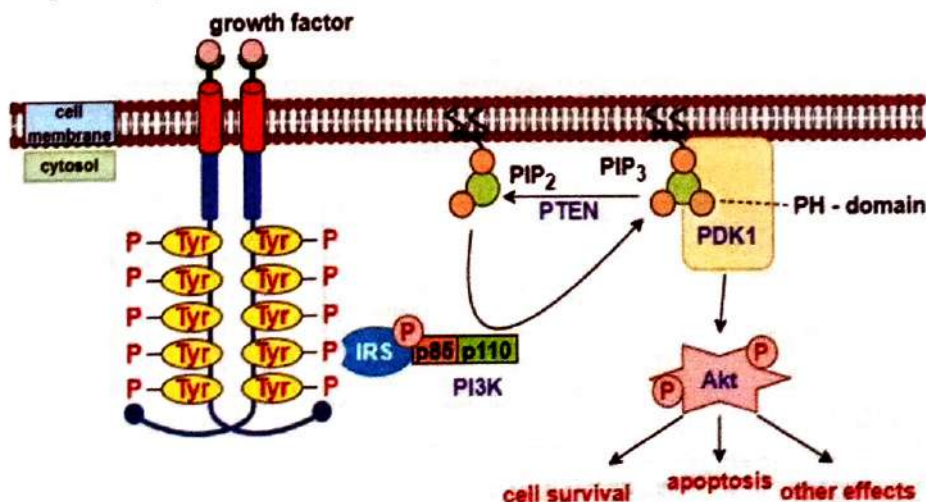


Fig. 5-3 Phosphatidylinositol-3-kinase (PI3K) pathway

mTOR as a key modulator of aging and age-related diseases

mTOR (mammalian target for rapamycin) is a serine/threonine protein kinase that is part of the downstream PI3K/Akt pathway. **mTOR** regulates *cell growth*, *proliferation*, *mobility*, *cell survival*, *protein biosynthesis* and *metabolism*. **Dysregulation of mTOR** signaling contributes to tumorigenesis, as it promotes anabolic processes, including protein synthesis and lipid metabolism, while inhibiting autophagy.

Rapamycin (sirolimus) is a bacterial macrolide antibiotic that inhibits mTORC1 by association with its intracellular receptor. mTOR serves as the **catalytic subunit** of two molecular complexes: **mTORC1 and mTORC2**.

1. mTORC1 (mTOR complex 1) as a nutrient and energy sensor: sensitive to rapamycin, regulates protein synthesis, lipid metabolism, autophagy suppression. mTORC1 is activated by PI3K/Akt in response to insulin and growth factors (IGF-1, IGF-2, PDGF, EGF, HGF). mTORC1 is activated by sufficient amino acids, ATP levels, and oxygen. It is inhibited under energy stress (low ATP via AMPK activation) or hypoxia. Inhibition of mTORC1 can reduce tumor growth and is targeted in cancer therapies (e.g., rapamycin analogs).

2. mTORC2 (mTOR complex 2): does not respond directly to nutrients but is involved in cytoskeletal regulation and cell survival (via Akt phosphorylation at Ser473).

2. Lipid mediators formed from sphingolipids - ceramide and sphingosine - 1 phosphate (S1P)

Ceramide is a second messenger in apoptosis.

Ceramide is indeed a key **bioactive sphingolipid** involved in apoptosis initiation, particularly *in response to cellular stress* (e.g., TNF signaling, oxidative stress, radiation, heat shock). It *regulates mitochondrial function and death receptor pathways to induce apoptosis*.

Ceramide can regulate cell death by two different mechanisms (Fig. 5-4):

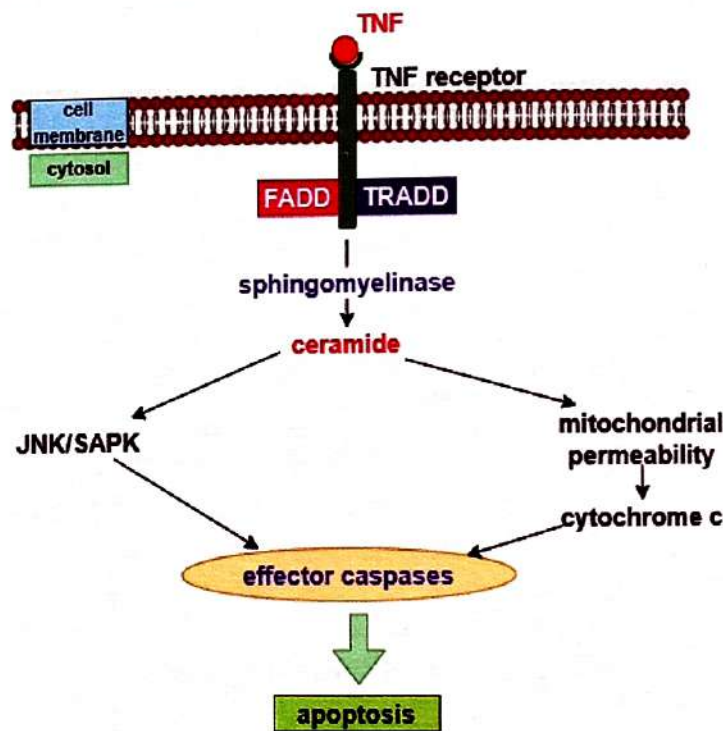


Fig. 5-4 Action of ceramide as a secondary mediator

1. Extrinsic (receptor-mediated) pathway – JNK dependent

- TNF- α , Fas (CD95), and other death receptor pathways lead to ceramide formation.
- Ceramide facilitates **JNK activation**, leading to pro-apoptotic transcription via **FADD** (FAS-associated death domain protein), **TRADD** (tumor necrosis factor receptor type 1-associated DEATH domain protein), and the **death domain (DD)** of TNF receptors.

This can also **activate caspase cascades**, reinforcing apoptotic signaling.

JNK (c-Jun N-terminal kinase) is part of the mitogen-activated protein kinase (MAPK) family and plays a role in regulating cell death, inflammation, and differentiation. Its dysregulation is linked to various diseases, including *cancer and neurodegenerative disorders*.

2. Intrinsic (mitochondria-mediated) pathway

- Ceramide is involved in **mitochondrial permeability**, leading to **cytochrome c release** and activation of **caspase-9 and caspase-3**.
- It can be generated in response to **UV radiation, ROS, and ionizing radiation** without direct receptor signaling.

Niemann-Pick disease and ceramide deficiency

- In **Niemann-Pick disease (types A and B)**, a deficiency in **acid sphingomyelinase (ASM)** results in **reduced ceramide production**, potentially impairing apoptosis.
- This could contribute to altered cell survival mechanisms.
- Niemann-Pick disease primarily leads to **lipid accumulation and neurodegeneration**—the role of ceramide deficiency in apoptosis inhibition is not fully conclusive.

Sphingosine-1-phosphate (S1P) as an anti-apoptotic mediator

- S1P is produced by **sphingosine kinase (SPHK)** and promotes **cell survival, proliferation, migration, and angiogenesis**.
- External stimuli such as **growth factors (PDGF, VEGF, TNF- α , NGF, EGF, bFGF), vitamin D3, and ATP** activate SPHK to generate S1P.
- S1P can function in an autocrine or paracrine manner by stimulating S1P receptors. This activates various G-proteins and multiple signaling pathways. Sphingosine kinase produces S1P, which is **stored** in the cell and/or **exported** in the extracellular environment.

S1P and Jak/STAT & NF- κ B pathways (Fig. 5-5)

- **Extracellular S1P** binds to **S1P receptors (S1PRs)**, activating **Jak/STAT3**, leading to **IL-6 and inflammatory signaling**.
- **Intracellular S1P** can activate **NF- κ B** (nuclear factor kappa B), promoting cell survival.

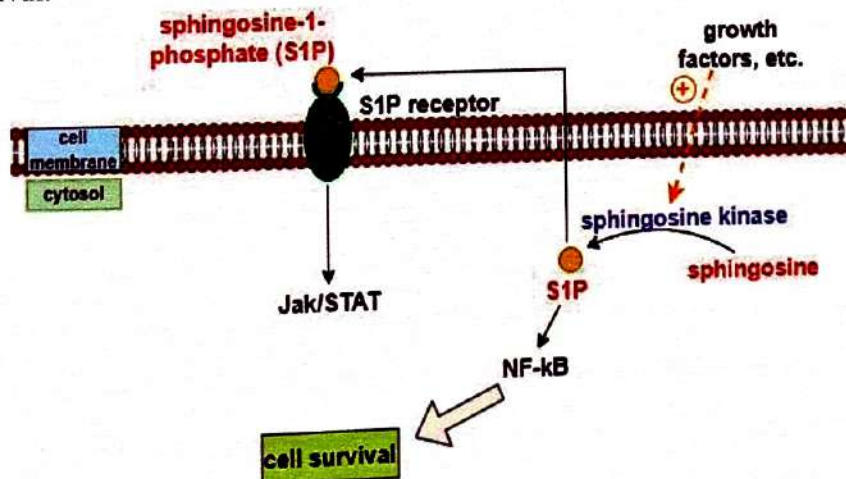


Fig. 5-5 Sphingosine-1-phosphate pathway

Ceramide/S1P ratio as a "Molecular Rheostat" regulating cell fate

- The **balance between ceramide (pro-apoptotic) and S1P (pro-survival)** is often described as a **"rheostat"** that determines cell fate.
- High **ceramide** → **apoptosis**, high **S1P** → **survival, proliferation, and tumorigenesis**.



6. Hormones

Endocrine glands or endocrine cells dispersed in other organs synthesize and release hormones, which are transported through the bloodstream to their target cells. Only cells that have the **appropriate receptors** can respond to these hormones.

A hormone is any substance in the body that transmits a signal and leads to a change at the cellular level (a change in cellular behavior as a biological response).

Classification of hormones and signaling molecules based on their chemical structure

1. **Peptide and protein hormones** *Water Soluble*
 - Composed of chains of amino acids.
 - *Examples:* Insulin, Glucagon, Growth Hormone (GH), Adrenocorticotrophic Hormone (ACTH).
2. **Steroid hormones** *Lipid Soluble*
 - Derived from cholesterol.
 - **Lipid-soluble** and can cross cell membranes.
 - *Examples:* Cortisol, Aldosterone, Estrogens (e.g., Estradiol), Androgens (e.g., Testosterone).
3. **Amino acid-derived hormones**
 - Synthesized from single amino acids, primarily tyrosine or tryptophan.
 - *Examples:* **Thyroid Hormones (T3, T4)**, Catecholamines (Epinephrine (adrenalin), Norepinephrine (noradrenalin), Dopamine), Melatonin.
4. **Lipid-derived hormones (eicosanoids)** *Lipid Soluble*
 - Derived from fatty acids, primarily arachidonic acid.
 - *Examples:* Prostaglandins, Leukotrienes, Thromboxanes.
5. **Glycoprotein hormones**
 - Consist of protein chains with carbohydrate groups attached.
 - *Examples:* Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH), Thyroid-Stimulating Hormone (TSH).
6. **Gaseous signaling molecules**
 - Small, diffusible gas molecules that function as signaling mediators.
 - Mechanism:
 - **Nitric Oxide (NO):** Acts as a vasodilator by activating guanylate cyclase, increasing cyclic GMP levels.
 - **Carbon Monoxide (CO):** Modulates cellular responses through heme oxygenase activity and impacts inflammation and apoptosis.
 - **Hydrogen Sulfide (H₂S):** Influences cardiovascular protection and neural signaling via sulfhydrylation of protein targets.

Mechanism of action for Nitric Oxide (NO), Carbon Monoxide (CO), and Hydrogen Sulfide (H₂S) as signaling molecules

1. Carbon Monoxide (CO)

Source: CO is produced endogenously during the degradation of heme by the enzyme *heme oxygenase (HO-1 and HO-2)* - see fig. 8-2 from Medical Biochemistry Part I.

Mechanism of Action:

- Inhibition of *cytochrome c oxidase*: CO binds to the heme group of *cytochrome c oxidase* (Complex IV) in the mitochondrial electron transport chain. This binding competitively inhibits oxygen binding, leading to a reduction in mitochondrial respiration and ATP production. The inhibition is reversible and *dose-dependent*, with physiological levels of CO modulating mitochondrial activity rather than completely halting it. By modulating mitochondrial respiration, CO can *reduce the production of reactive oxygen species (ROS)*, contributing to its *cytoprotective and anti-apoptotic effects*.
- Activates *soluble guanylate cyclase (sGC)*, leading to increased levels of **cyclic GMP (cGMP)**, which mediates smooth muscle relaxation, vasodilation, and other cellular effects.
- Modulates **inflammation** by inhibiting pro-inflammatory cytokines and promoting anti-inflammatory pathways.
- Regulates **apoptosis** and cell survival through interaction with mitochondrial proteins.

Physiological Effects: Vasodilation, anti-inflammatory effects, cytoprotection, and regulation of oxidative stress.

2. Hydrogen Sulfide (H₂S)

Source: H₂S is synthesized enzymatically from **L-cysteine** by the action of enzymes like *cystathionine γ -lyase (CSE)*, *cystathionine β -synthase (CBS)*, *3-mercaptopyruvate sulfurtransferase (3-MST)*. **Dietary Sources:** Sulfur-containing amino acids (e.g., cysteine and methionine) in protein-rich foods can contribute to H₂S production.

Mechanism of Action:

- **Sulfhydration of protein targets:** Modifies cysteine residues on proteins to form *persulfides*, altering their activity and function.
- Activates **ATP-sensitive potassium channels** in smooth muscle, leading to vasodilation.
- Modulates signaling pathways, such as the **NF- κ B pathway**, regulating inflammation and apoptosis.
- Reduces oxidative stress by increasing levels of antioxidant molecules like glutathione.
- Stimulates the production of **cyclic AMP (cAMP)** and interacts with mitochondrial electron transport chain components, improving energy metabolism.

Physiological Effects: Cardiovascular protection, anti-inflammatory effects, cytoprotection, neuromodulation, and regulation of oxidative stress.

3. Nitric Oxide (NO) - see chapter 5

Depending on their *solubility*, hormones are **hydrophobic** (easily crossing cell membranes, which are also hydrophobic) and **hydrophilic** (unable to cross membranes) (Table 6-1).

Table 6-1 Basic properties of hormones

	Group I	Group II
Types	<i>steroids, T3/T4, calcitriol, retinoic acid</i>	<i>polypeptides, proteins, glycoproteins, catecholamines</i>
Solubility	<i>hydrophobic, lipophilic</i>	<i>hydrophilic, lipophobic</i>
Transport Protein	<i>there is</i>	<i>there is no</i>
Plasma half life	<i>long (hours, days)</i>	<i>short (minutes)</i>
Receptor	<i>intracellular receptor</i>	<i>membrane receptor</i>
Secondary mediator	<i>hormone-receptor complex</i>	<i>cAMP, cGMP, Ca²⁺, inositol triphosphate (IP₃), and diacylglycerol (DAG).</i>

6.1 Pituitary hormones

Hypothalamus-pituitary system

Releasing hormones initiate a signaling pathway that is associated with Ca^{2+} release, IP_3 (inositol triphosphate), and PKC (protein kinase C) activation leading to exocytosis of synthesized in advance hormone-containing vesicles from the anterior pituitary.

The **tropic hormones** that are released from the pituitary gland have *peripheral endocrine glands* as their target organ.

The pituitary gland consists of **two parts**, both controlled by the hypothalamus:

1. **Adenohypophysis** or *anterior lobe*
2. **Neurohypophysis** or *posterior lobe*

There are 5 distinct endocrine axes in the hypothalamic-pituitary system (Fig. 6-1).

The first three axes are part of a complex three-level endocrine axis in which the pituitary hormones (TSH, ACTH and FSH/LH) are tropic hormones for the target organs (*thyroid, adrenal and gonads* respectively). In these axes, control is exerted through a cascade to ensure *amplification* of the content of the secreted hormone and its *biological activity* (half-life time).

The fourth endocrine axis is hybrid because *growth hormone* (GH) is both tropic and with another specific activity.

The fifth endocrine axis leads to the secretion of *prolactin*, which is not a tropic hormone.

There are various endocrine diseases associated with an *excess or deficiency* of hormones formed by the anterior lobe of the pituitary gland. Pituitary tumors can be *hormone-secreting* or *non-functioning*. In the first case, there is a *hormonal excess*, and in the second case there is a *hormone deficiency* since the tumor can affect and disrupt areas of hormone-secreting cells.

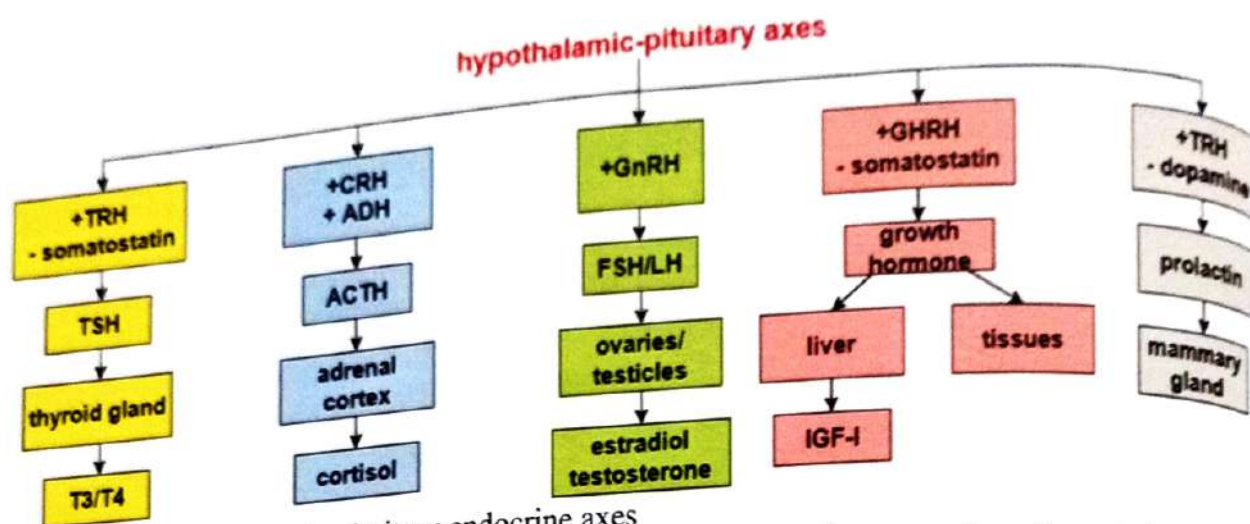


Fig. 6-1 Hypothalamic-pituitary endocrine axes
 I horizontal row: hypothalamic hormones; II horizontal row: hormones from the anterior lobe of the pituitary gland; III horizontal row: target organs; IV horizontal row: products; TRH - thyrotropin releasing hormone; TSH - thyroid stimulating hormone; T4/T3 - thyroxine/triiodothyronine; CRH - corticotropin releasing hormone; ADH - antidiuretic hormone; ACTH - adrenocorticotropic hormone; GnRH - gonadotropin releasing hormone; FSH/LH - follicle stimulating/uteinizing hormone; GHRH - growth hormone releasing hormone; GH - growth hormone; IGF-I insulin-like growth factor-I
 Releasing factors or **releasing hormones** are synthesized in the *hypothalamus*. They are synthesized in the *neurons* of the hypothalamus and secreted in the *axon terminals* in the portal circulation of the *adenohypophysis*.

6.1.1 Hormones from the anterior pituitary gland

In the **adenohypophysis**, five hormones are produced, known as *tropic hormones*, as they regulate the growth and hormonal secretion of target cells.

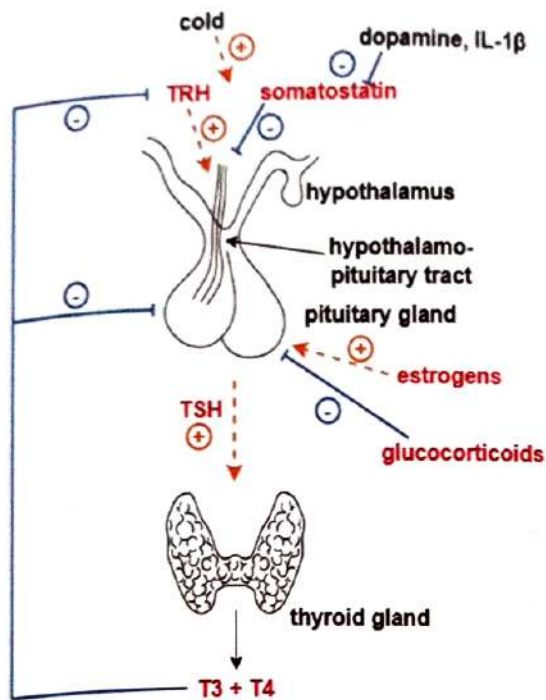
The five tropic hormones have the following molecular characteristics:

1. One group of hormones consists of **glycoproteins** with two peptide chains. They have specific, biologically **active β -chains** for each of the three hormones—**TSH, FSH, and LH**—while the **inactive α -chain** is identical for all.
2. **Growth hormone and prolactin** are gene duplicates of the same prohormone molecule. They have a polypeptide chain of ~ 200 amino acids with almost identical sequences.
3. **Pro-opiomelanocortin (POMC)** is a common precursor for hormones of the adenohypophysis—**ACTH, β -endorphin (endogenous opiate), β -lipotropin, α -MSH, and β -MSH (melanocyte-stimulating hormone)**, which are derived through specific proteolysis (see Fig. 2-1).

Hypothalamic-pituitary-thyroid axis (Fig. 6-2)

Thyrotropin-releasing hormone (TRH) is produced in the *hypothalamus* and transported through the portal circulation to the pituitary gland, where it triggers the exocytosis of **thyroid-stimulating hormone (TSH)**. TRH is a modified tripeptide, synthesized as a *prohormone* by the peptidergic hypothalamic nucleus and transported to the adenohypophysis.

TRH stimulates the *biosynthesis of the subunits of TSH and its glycosylation*. The number of TRH receptors is controlled through a negative feedback mechanism based on the concentration



of TSH itself and thyroid hormones. At the pituitary level, T4 and T3 decrease the synthesis and secretion of TSH by regulating gene transcription and the glycosylation of TSH.

Fig. 6-2 Hypothalamic-pituitary-thyroid axis

Key signaling pathways

Activation of the **TRH receptor** leads to the stimulation of **phospholipase C**. The generated **inositol triphosphate** activates the release of *calcium* from its intracellular stores, and this results in the **exocytosis of TSH**.

The activated **calcium/calmodulin-dependent protein kinase, PKC, and MAPK** induce gene transcription through the transcription factors **CREB** (cAMP response element-binding protein), **AP-1** (Activator protein 1), and **Elk-1** (ETS Like-1 protein).

The **TSH receptor** is a glycoprotein with an extracellular domain, 7 transmembrane regions, and an intracellular domain. The binding of TSH to the receptor activates **adenylate cyclase/phospholipase C**, with secondary messengers being **cAMP/lipid mediators** (Fig. 6-3).

TSH influences every aspect of thyroid hormone synthesis and secretion: *iodide uptake, thyroglobulin synthesis, iodination of tyrosine residues, coupling of iodotyrosines, and endocytosis of thyroglobulin, followed by proteolytic cleavage to release T3 and T4 into the bloodstream. It also regulates thyroid cell functions and growth.*

T4 is quantitatively the most significant thyroid hormone and is produced exclusively in the thyroid gland. T3 is *the biologically active form*, derived through **5'-deiodination** of T4. This process can occur in the thyroid gland, target tissues, or other peripheral tissues. The removal of iodine at the 5-position instead of the 5'-position results in reduced biological activity, producing the so-called "reverse" T3. Thus, **the control of deiodination** is a way to regulate the activity of thyroid hormones.

On the basal side of the cell is the **NIS (sodium-iodide symporter)**. It transports iodide (I^-) using the *sodium gradient*. Iodide is transported through the protein **pendrin** across the apical membrane into the follicular lumen. There, it is oxidized by the enzyme **thyroid peroxidase**, which is activated by **TSH**. The granular endoplasmic reticulum synthesizes **thyroglobulin**, a protein rich primarily in tyrosyl residues, and it is secreted into the lumen. With the help of thyroid peroxidase again, the tyrosyl residues undergo **iodination**. Initially, **monoiodotyrosine** is formed, followed by **diiodotyrosine**. The combination of one mono- and one diiodotyrosine

leads to the formation of **triiodothyronine**. The combination of two diiodotyrosines results in the formation of **tetraiodothyronine**.

Through **phagocytosis** or **micropinocytosis**, the **colloid** is taken up by the thyrocyte via an **endosome**. It then fuses with the lysosome, leading to the production of **100% of T4 and 10% of T3** in the thyroid gland. The activity of thyroid hormones is regulated by the conversion of T4 into T3 through deiodination (see Fig. 5-19 and 5-20 from Medical Biochemistry Part I). The biologically active fraction of T3 and T4 in plasma is the unbound form, representing less than 1% of the total amount—0.02% for T4 (FT4) and 0.3% for T3 (FT3).

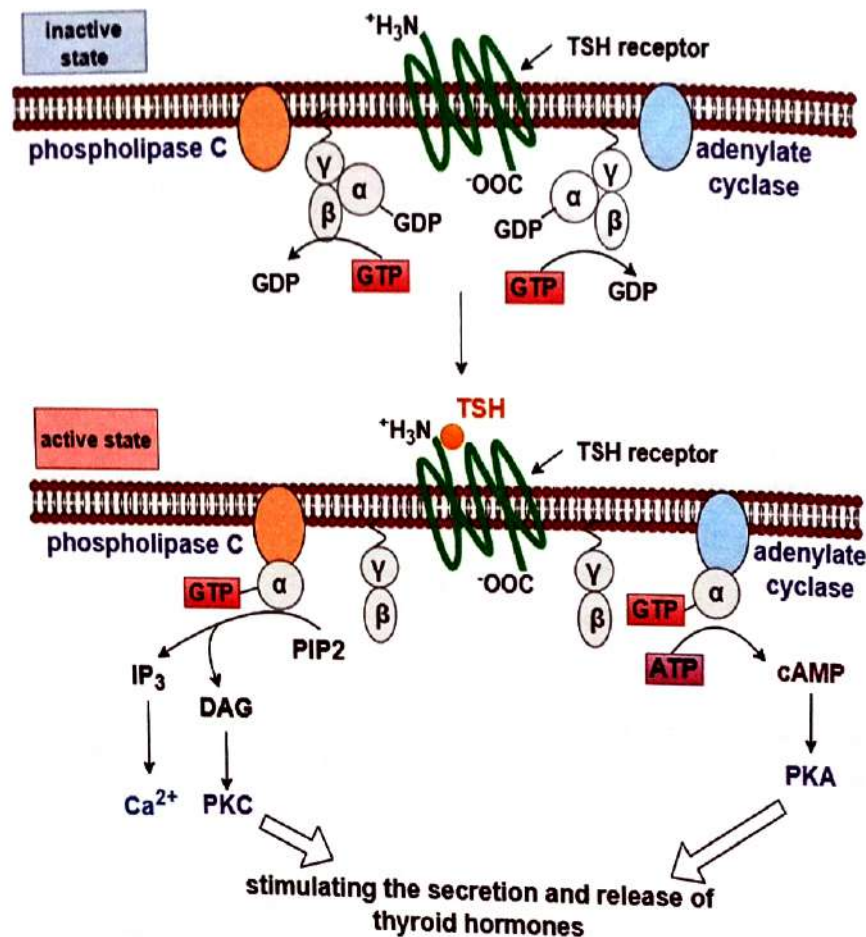


Fig. 6-3 Mechanism of action of TSH

Effects of thyroid hormones (THs) on metabolism

Lipid Metabolism: THs, particularly triiodothyronine (T3), stimulate **lipolysis** by activating **hormone-sensitive lipase (HSL)** in adipocytes, leading to increased mobilization of free fatty acids (FFAs) into the bloodstream. Additionally, T3 **enhances β -oxidation of fatty acids** in the liver and skeletal muscles by upregulating the expression of enzymes involved in mitochondrial lipid oxidation.

Carbohydrate Metabolism: THs **enhance all major aspects of carbohydrate metabolism**, including:

- **Increased gluconeogenesis** – they activate key enzymes such as *phosphoenolpyruvate carboxykinase (PEPCK)* and *glucose-6-phosphatase* in the liver, leading to enhanced glucose synthesis.

- **Glycogenolysis** – they promote glycogen breakdown in the liver by increasing *glycogen phosphorylase* activity, thus raising free glucose levels.

Stimulation of Metabolic Rate: In most tissues (with exceptions including the brain, spleen, and testes), THs stimulate the metabolic rate by:

1. Increasing the **number and size of mitochondria**;
2. Stimulating the synthesis of **respiratory chain enzymes**;
3. Increasing the membrane concentration of Na^+/K^+ ATPase and membrane **permeability to Na^+ and K^+** .

Between 15% and 40% of *resting cellular energy* is used to maintain membrane gradients, mainly through sustaining Na^+/K^+ ATPase activity.

THs are key regulators of **basal metabolic rate** (BMR). BMR can increase by up to 100% with excess thyroid hormones or decrease by up to 50% in cases of hormone deficiency.

THs stimulate various metabolic activities in tissues, leading to an **increase in basal metabolic rate** and an **increase in body heat production** due to higher oxygen consumption and ATP hydrolysis rates. THs maintain BMR by **uncoupling oxidative phosphorylation in mitochondria** or by reducing the activity of shuttle systems that transfer hydrogen into the mitochondria.

Resting energy expenditure (REE), which represents the energy needs of major organs such as the liver, brain, heart, and kidneys, is highly sensitive to THs.

(In practice, BMR and REE differ by no more than 10%—REE is typically slightly higher—and the terms are often used interchangeably.)

Disorders of thyroid functions

1. Thyroid dysfunction affects nearly **3% of the population** and is 9 times more common in women.
2. Thyroid diseases occur at **every stage of ontogenesis**.
3. More than 95% of thyroid diseases are related to disorders of the thyroid gland, and most have an **autoimmune nature**, involving the formation of autoantibodies.

A. If the autoantibodies bind but do not stimulate hormone production, hormone levels will decrease, and the patient will suffer from **hypothyroidism**, with **increased plasma TSH levels and reduced free T4**.

B. If the autoantibodies stimulate the production of thyroid hormones, the patient will suffer from **hyperthyroidism (thyrotoxicosis)**, with **elevated plasma T4 and suppressed TSH**.

4. **Hypothalamic and pituitary disorders** can cause hypothyroidism due to suppression of TSH secretion by an adjacent tumor. Pituitary tumors that secrete TSH are also known, but they are rare.

Hyperthyroidism – a **hypermetabolic state** characterized by increased resting energy expenditure (REE), weight loss, reduced cholesterol levels, increased lipolysis, and enhanced gluconeogenesis.

Hypothyroidism – a **hypometabolic state** with reduced REE, weight gain, increased cholesterol levels, decreased lipolysis, and reduced gluconeogenesis.

Hypothalamic-pituitary-adrenal axis

Hormonal control of Steroid Hormone (StH) synthesis

The specific class of StH produced by a cell depends on the *expression of receptor types*. Steroid hormone synthesis is under hormonal control from the *pituitary gland*

1. **Luteinizing hormone (LH):**

- In **females**, LH stimulates the **theca cells** in the ovaries to produce **androgens**, which are later converted into **estradiol** by granulosa cells. It also triggers **progesterone production** by the corpus luteum after ovulation.
- In **males**, LH stimulates **Leydig cells** in the testes to produce **testosterone**.

2. **Adrenocorticotrophic hormone (ACTH):** ACTH stimulates the **zona fasciculata** of the adrenal cortex to synthesize and secrete **cortisol**. It also has a minor effect on **androgen production** in the zona reticularis.

3. **Follicle-stimulating hormone (FSH):**

- In **females**, FSH primarily stimulates **granulosa cells** to promote follicular growth and the conversion of androgens (from theca cells) into **estradiol** via **aromatase**. However, LH is also essential for estradiol synthesis by providing the androgen precursors.
- In **males**, FSH stimulates **Sertoli cells**, supporting **spermatogenesis** and the production of **inhibin B**, which regulates FSH secretion.

4. **Angiotensin II/III – regulates the synthesis of aldosterone:**

- **Angiotensin II (not III)** is the primary regulator of **aldosterone synthesis** in the **zona glomerulosa** of the adrenal cortex.
- It acts via the **angiotensin II type 1 receptor (AT₁R)**, stimulating the enzyme cascade leading to **aldosterone production**.
- **Potassium levels** and **ACTH** also play minor regulatory roles in aldosterone secretion.

Steroid Hormones

1. They are not synthesized and stored in advance but are *produced at the moment of the stimulus*.
2. **Cholesterol** is the substrate for all steroid hormones.
3. The enzymes involved in their synthesis are localized in the **mitochondria** and the smooth endoplasmic reticulum.
4. StH are **lipophilic (hydrophobic)**, allowing them to freely cross membranes.
5. Since they are water-insoluble, they require protein carriers in the blood. For example, **corticosteroid-binding globulin** carries cortisol, **sex hormone-binding globulin** transports testosterone and estradiol, and **albumin** acts as a nonspecific carrier.
6. With the exception of aldosterone, all steroid hormones undergo both **central metabolism** (in the tissue that synthesizes them) and **peripheral metabolism** (in the CNS, muscles, skin, adipose tissue, etc.), where their activity may increase or inactive forms may become active.
7. They play roles in:
 - Regulation of **carbohydrate metabolism** – glucocorticoids;
 - **Mineral balance** – mineralocorticoids;
 - **Reproductive functions** – sex hormones.

8. Other functions include involvement in *inflammatory responses, stress, bone metabolism, behavior, mood, cognitive functions, and more.*

All steroid hormones are synthesized from cholesterol. Sources of cholesterol include **acetyl-CoA** (in *de novo* synthesis) and **lipoproteins**.

Cholesterol transport is facilitated by **StAR** (**steroidogenic acute regulatory protein**) in the mitochondria. StAR is located on the outer mitochondrial membrane, where cholesterol precursor accumulates. StAR contains an internal domain called the **StAR-related lipid transfer domain (START)**, which facilitates cholesterol shuttling through the aqueous intermembrane space to reach the **side-chain cleavage enzyme** located in the inner mitochondrial membrane.

Steroid synthesis begins with the cleavage of the side chain between **C20 and C22** of cholesterol, producing **pregnenolone**. The enzyme responsible for this reaction functions with **cytochrome P450** and **NADPH**, utilizing a *short electron transport chain* to hydroxylate carbon atoms before cleavage. This enzyme is known as the *side-chain cleavage enzyme (scc)* or *desmolase*, and it is the **main** regulatory enzyme. The reaction takes place in the mitochondria.

Biosynthesis of mineralocorticoids - in the *Zona glomerulosa* (fig. 6-4)

1. ***3 β -Hydroxysteroid dehydrogenase: $\Delta^{5,4}$ isomerase*** – This enzyme recognizes the hydroxyl group at the 3rd position, converting it into a keto group, and shifts the double bond from position 5-6 to position 5-4. The reaction is reversible. The enzyme is present in all three zones of the adrenal cortex (as isoenzymes). The product of this reaction is **progesterone**.
2. ***21-Hydroxylase*** – Catalyzes the hydroxylation at position 21 with the participation of cytochrome P450. The product of this reaction is **11-deoxycorticosterone**.
3. ***11 β -Hydroxylase*** – Catalyzes the hydroxylation at position 11 with the participation of cytochrome P450. The product of this reaction is **corticosterone**.
4. ***Aldosterone synthase*** – Introduces an aldehyde group at position 18, leading to the formation of the mineralocorticoid **aldosterone**.

The primary action of aldosterone occurs in the **kidney**, but it also affects the **salivary glands** and **colon**. It acts on a specific **Na⁺ channel** by stimulating the gene expression of **ENaC (epithelial sodium channel)** mRNA, *enhancing sodium reabsorption*, which in turn promotes *water reabsorption*. In addition to the adrenal cortex, **local aldosterone synthesis** occurs in various tissues.

3. **21-Hydroxylase (a different isoform)** – Hydroxylation at position 21, producing **11-deoxycortisol**.
4. **11 β -Hydroxylase** – Hydroxylation at position 11, resulting in **cortisol**.

Cortisol shares structural similarities with aldosterone, which explains their overlapping effects. The **cortisol/aldosterone ratio** is **100:1**.

Cortisol undergoes extensive **peripheral metabolism**. All aldosterone-sensitive tissues (such as the **intestines and salivary glands**) contain the enzyme **11 β -hydroxysteroid dehydrogenase (11 β HSD)**, which converts the *hydroxyl group at position 11* into a *keto group*, inactivating cortisol into **cortisone**.

In contrast, other tissues (**liver, skin, adipose tissue**) contain a different **isoform** of this enzyme, which **reverses the reaction**—converting **cortisone back into cortisol**, thereby reactivating it (Fig. 6-5).

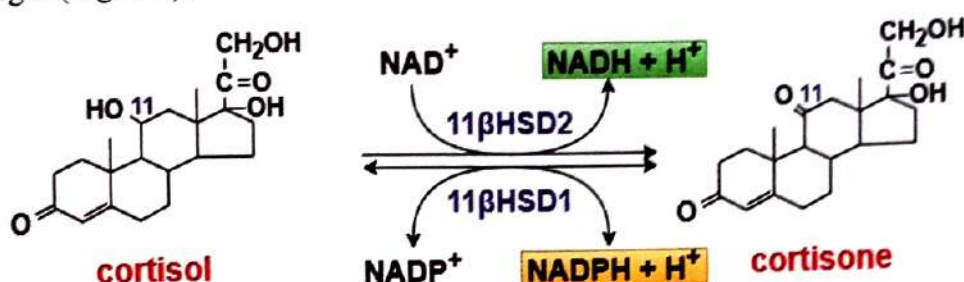
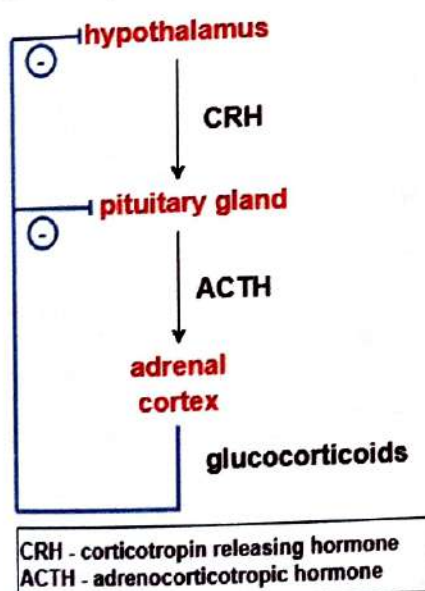


Fig. 6-5 Peripheral cortisol metabolism

Negative feedback regulation (Fig. 6-6)

Cortisol exerts control at the level of the **pituitary gland and hypothalamus** through **negative feedback regulation**, inhibiting the production of **CRH** (corticotropin-releasing hormone) and **ADH** (antidiuretic hormone, vasopressin).



- **Rapid negative feedback** – Inhibits the release of hypothalamic CRH and CRH-mediated ACTH secretion.
- **Slow negative feedback** – Results from the reduced synthesis of CRH and ADH, along with the suppression of POMC gene transcription, leading to decreased ACTH synthesis.

Fig. 6-6 Negative feedback regulation

Releasing hormones - CRH and ADH

Two hypothalamic peptides are the **primary, but not the only, regulators** of **pituitary synthesis and secretion**. They act **independently and synergistically**.

- **CRH** is a **41-amino acid peptide**, secreted by the **paraventricular nucleus**, which signals through the **cAMP system**.

- **ADH** consists of **9 amino acids** and is synthesized by the **supraoptic and paraventricular nuclei**. ADH acts synergistically with CRH on the anterior pituitary via **V_{1b} (V₃) receptors**, which signal through intracellular calcium mobilization (PLC-IP₃-Ca²⁺ pathway).

Peptides formed during the cleavage of POMC - see Fig. 2-1

Adrenocorticotrophic hormone (**ACTH**) is derived from a **285-amino acid precursor** called **pro-opiomelanocortin (POMC)**.

The **POMC gene** is expressed in the **anterior and intermediate lobes** of the pituitary gland.

The **primary protein product** of the POMC gene undergoes **various processing steps (proteolysis)**, leading to the formation of at least **eight peptides**, depending on the **site of synthesis and the stimuli**. Initially, **ACTH** and **β -lipotropin** are produced, and further proteolytic cleavage of these peptides generates other **biologically active peptides**.

- **β -lipotropin** is a **90-amino acid polypeptide**, derived from the **C-terminal region** of POMC. It stimulates **lipolysis** and **steroidogenesis**, promotes **melanin production** by melanocytes, and can be further cleaved into **smaller peptides**.

Further proteolytic cleavage of ACTH leads to the formation of:

- **α -Melanocyte-Stimulating Hormone (α -MSH)**
- From **β -lipotropin**, additional peptides are produced: **γ -lipotropin, β -MSH, α -endorphin, β -endorphin, γ -endorphin, and met-enkephalin**.
- **Endorphins** ("endogenous morphine") are endogenous opioid peptides that function as neurotransmitters. They are derived from POMC during physical exercises, stress, fear, excitement, pain, love, and orgasm. They have analgesic effects and induce a sense of well-being.
- **Melanocyte-Stimulating Hormones (MSH)** stimulate the synthesis and release of melanin from melanocytes in the skin and hair and also play a role in appetite regulation.

ACTH secretion and effects of cortisol

Adrenocorticotrophic hormone (**ACTH**) is secreted under **stressful conditions** and follows a **circadian rhythm**, with a peak at **5:00 AM**. Its **half-life** is **10 minutes**, and it is unstable in plasma.

ACTH stimulates the **synthesis and release of glucocorticoids** by interacting with **membrane receptors** on the **adrenal cortex**, leading to an **increase in cAMP production**.

Regulation of mineralocorticoid synthesis in the adrenal cortex – via the renin-angiotensin-aldosterone system (see Fig. 7-1)

Effects of cortisol

1. **Carbohydrate metabolism** – Cortisol activates **gluconeogenesis**. At the same time, it inhibits glucose uptake and metabolism in peripheral tissues.
2. **Lipid metabolism** – It stimulates **lipolysis**.
3. **Protein metabolism** – Cortisol stimulates **protein and RNA synthesis in the liver** but **inhibits them in peripheral tissues (muscles)**, where proteins are degraded. The amino acids released serve as substrates for gluconeogenesis. In this role, cortisol works in tandem with other hormones, including glucagon and growth hormone.

4. Excess cortisol has a wide range of effects on the **immune system**, causing **general suppression**, which makes it useful as a **therapeutic agent**.
5. Cortisol also influences the **heart, blood vessels, blood pressure, water excretion, and electrolyte balance**. While some of these effects are **mineralocorticoid-like**, others are **mediated through cortisol receptor activation**.
6. Cortisol affects **bone metabolism** through **multiple mechanisms**. Osteoporosis is a well-known consequence of **chronic cortisol excess** (such as from **prolonged glucocorticoid therapy**).

Finally, cortisol can **inhibit linear growth and cell division** in certain tissues.

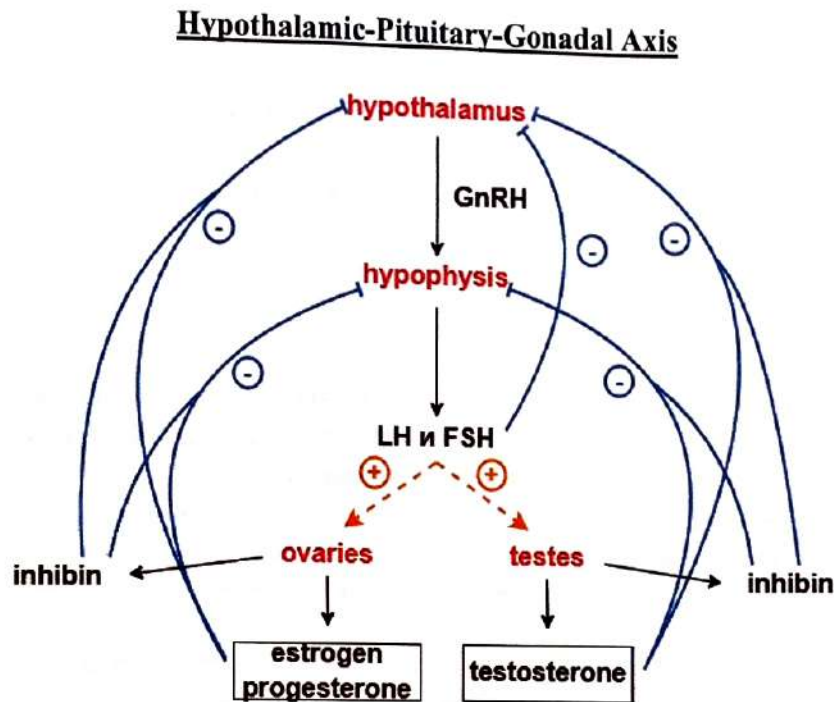


Fig. 6-7 Hypothalamic-Pituitary-Gonadal Axis

Gonadotropin-releasing hormone (GnRH)

The hypothalamus plays a central role in controlling reproductive function in both men and women. Although follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were named based on their roles in women, these hormones are also produced and function in men.

The secretion of GnRH marks the *first stage of puberty*.

GnRH is a 10-amino acid peptide, produced by various hypothalamic nuclei and transported to the pituitary gland via the portal system. It has a half-life of approximately 3 minutes and stimulates the synthesis of **FSH and LH**.

GnRH acts via a membrane receptor, leading to activation of *phospholipase C* (PLC), an increase in intracellular Ca^{2+} levels, and activation of *protein kinase C* (PKC).

Regulation of sex hormone synthesis

The synthesis of sex hormones is regulated by **pituitary hormones**.

- **FSH and LH** activate *desmolase* via the *adenylate cyclase* and *PKA* pathway.
- Although **many steroids** are produced in the **testes and ovaries**, the two most important are **testosterone** and **estradiol**.

- These hormones, in turn, regulate the secretion of FSH, LH, and GnRH through **negative feedback mechanisms**.
- This negative feedback can be **short-term or long-term**.
- **Low levels of circulating sex hormones** reduce the **inhibition of GnRH synthesis** (long-term negative feedback), leading to an **increase in FSH and LH secretion**.

Biosynthesis of sex hormones - in the *Zona Reticularis*

Before puberty and after menopause, the **adrenal glands** are the **primary source** of sex hormones.

- During **reproductive maturity**, the **gonads** become the **main source**.
- Women predominantly produce **estrogens** but also **require testosterone**.
- This hormonal balance changes **across different life stages** in both sexes.
- The **biosynthetic pathways** differ across tissues, starting from either **progesterone** or **pregnenolone**.

Key enzymes in sex hormone synthesis

1. **C-17,20 Lyase** – After hydroxylation at position 17, it cleaves the bond between C17 and C20, forming DHEA (dehydroepiandrosterone).
 - DHEA can be **sulfated** at position 3 (**DHEAS**), making it **water-soluble** and allowing it to circulate to peripheral tissues (**breast, bones, teeth, CNS, fat tissue**) for **local synthesis** of sex hormones.
 - In **women**, DHEA is an **essential source of estrogens**, providing **~75% of estrogens before menopause** and **100% after menopause**.
2. **3 β -Hydroxysteroid Dehydrogenase: $\Delta^5,4$ Isomerase** – Converts DHEA into androstenedione.
3. **17 β -Hydroxysteroid Dehydrogenase** – Converts the keto-group at position 17 into a hydroxyl group, forming androstenediol and testosterone.
4. **5 α -Reductase** – In many peripheral tissues, testosterone undergoes further metabolism.
 - 10% of testosterone is converted into the much more potent **5 α -dihydrotestosterone (DHT)**.
 - DHT has three isoenzymes and is crucial for **hair growth and sexual potency**.
 - It is **active in multiple tissues** in both **men and women** (testes, ovaries, prostate, skin, bones, CNS, etc.).

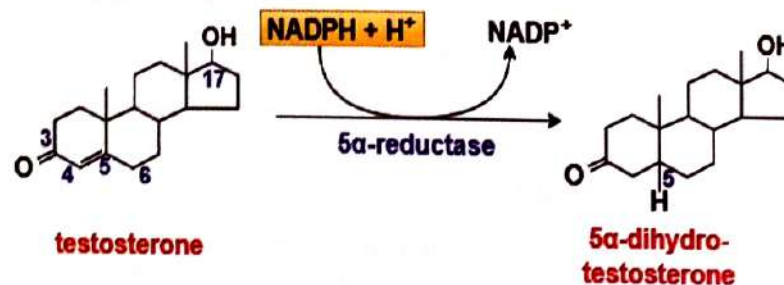


Fig. 6-8 Conversion of testosterone to 5 α -dihydrotestosterone

5. **Aromatase Enzyme System** – Converts **testosterone into estradiol**.
 - Aromatase makes the first steroid ring aromatic and removes the methyl group at position 19.
 - Testosterone is primarily metabolized into estradiol in peripheral tissues, such as **adipose tissue, CNS, and bones**.

- Locally synthesized estrogens in different organs have distinct functions (e.g., in bones, they help maintain bone density, preventing osteoporosis).

Clinical significance of sex hormone metabolism

- Many breast, ovarian, uterine, and prostate tumors are **hormone-dependent**.
- Aromatase inhibitors** are a class of drugs used to treat estrogen-sensitive breast cancer in postmenopausal women and gynecomastia in men.

After menopause, the ovaries cease to be the primary source of estrogen due to *follicular depletion*. However, estrogen synthesis *does not stop entirely*; instead, it continues through **peripheral conversion in various tissues, including adipose tissue, bones, skin, and the brain**.

The main estrogen produced postmenopausally is **estrone (E₁)**, which is derived from androstenedione, a steroid precursor secreted by the adrenal glands. This conversion occurs primarily through the action of **aromatase**, an enzyme found in **adipose tissue, bone**, and other peripheral tissues. In some tissues, estrone can be further converted into **estradiol (E₂)**, although in much lower amounts compared to premenopausal levels.

Growth Hormone Axis (Fig. 6-9)

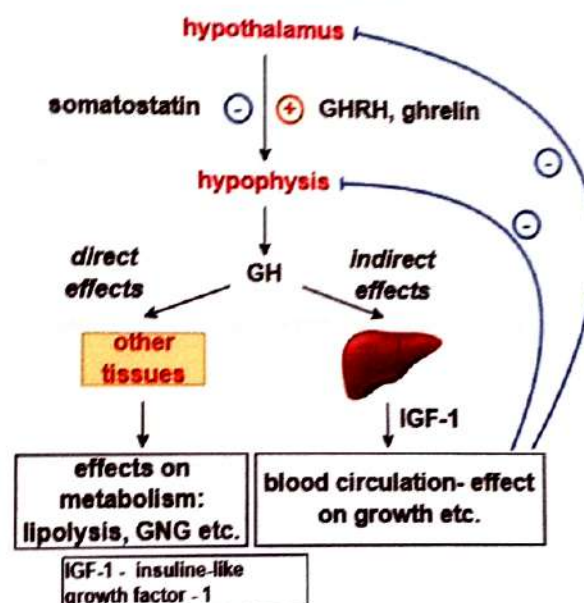


Fig. 6-9 Growth Hormone axis

Growth hormone (GH) and its regulation

GH and **prolactin** belong to the **same superfamily** of hormones. This family also includes **placental lactogens** (chorionic somatomammotropins, CS), also referred to as human placental lactogen (hPL).

GH secretion is regulated by:

- Growth Hormone-Releasing Hormone (GHRH)
- Somatostatin
- Hormones (such as estradiol, ghrelin)

- Metabolic fuels (such as **glucose** – inhibits GH secretion (negative feedback), **free fatty acids** (FFAs) – suppress GH release, **amino acids** (especially arginine) – stimulate GH secretion.)

These factors act on higher centers to **modify the secretion pattern of GHRH and somatostatin**.

Biochemical actions of Growth Hormone

Binding of **GH** to its receptors triggers a series of intracellular events, leading to the transcription of numerous **enzymes, hormones, and growth factors**, including **Insulin-like Growth Factor-1 (IGF-1)**.

The **diversity of GH's effects** makes it difficult to fully understand all its functions. Generally, GH has two main types of effects:

1. Direct Effects of GH

GH directly affects **carbohydrate and lipid metabolism**:

- GH's effects are **synergistic** with **cortisol** (stimulating **gluconeogenesis and lipolysis**).
- GH's effects **oppose** those of **insulin and IGF-1** (which promote glucose uptake and lipogenesis).

GH secretion is **stimulated in response to hypoglycemia**.

- GH *reduces glucose uptake* in certain tissues (e.g., muscles). This effect is indirect, as GH mobilizes free fatty acids from triglycerides in adipose tissue. Free fatty acids inhibit glucose uptake in muscle tissue.
- Chronic GH administration can lead to *diabetes* because prolonged hyperglycemia forces β -cells to *continuously secrete insulin*, leading to their eventual *exhaustion*.

2. Indirect Effects of GH

These are mediated by **several growth factors**, known as **somatomedins** or **Insulin-like Growth Factors (IGFs)**, primarily **IGF-1**.

GH effects include:

- Initiation of **protein biosynthesis**;
- **Cell proliferation** in **skeletal and non-skeletal tissues**;
- These effects are **insulin-like metabolic effects (opposite to cortisol's effects)**.

While GH is mainly known for stimulating *linear growth*, it also plays a major role in regulating the relative distribution of metabolites in adipose and muscle tissues. This is evident in GH-deficient patients undergoing GH replacement therapy.

Insulin-like Growth Factor-1 and -2 (IGF-1, IGF-2)

- IGF-1 is a 70-amino acid peptide.
- ~75% of IGF-1 is synthesized in the liver in response to GH stimulation.
- IGF-1 has a *structural homology to proinsulin*.

In plasma and extracellular fluids, IGF-1 binds to **IGF-binding proteins (IGFBPs)**, forming complexes.

IGF-1 acts via the Type 1 IGF receptor, which is:

- Structurally similar to the insulin receptor
- Possesses intracellular **tyrosine kinase activity**

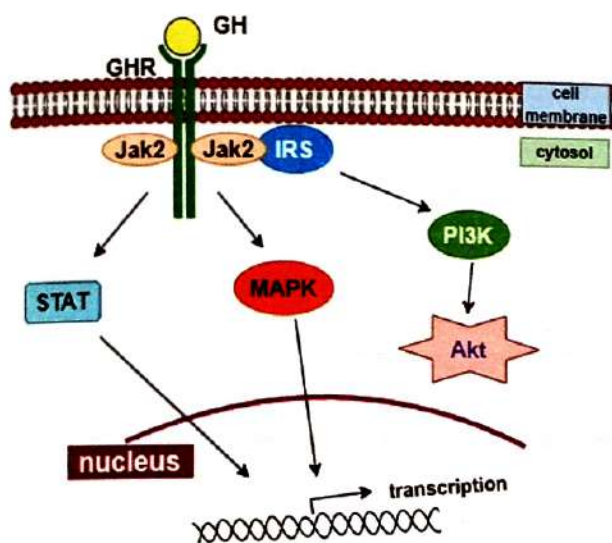
Under *normal physiological conditions*, cross-talk between insulin and IGF-1 receptors is minimal. However, under *pathological or pharmacological conditions*, insulin can act via the IGF-1 receptor and vice versa.

This cross-reactivity allows the potential use of IGF-1 as a therapeutic agent in Type 2 diabetes.

Key GH signaling pathways (Fig. 6-10)

GH binds to two receptor molecules (forming a dimer).

- The intracellular part of the receptor lacks enzymatic activity but activates **tyrosine kinases (Jak2)**.
- Jak2 kinases phosphorylate transcription factors, modulating gene expression.



GH activates multiple signaling pathways, including:

- **Jak2/STAT pathway** (see Fig. 4-11)
- **MAPK pathway** (see Fig. 4-6)
- **IRS/PI3K pathway** (see Fig. 6-2), which induces **insulin-like effects** of GH.

Fig. 6-10 Growth hormone signaling pathways

Disorders of GH production and signaling

GH Deficiency

- **GH-deficient dwarfism** results from the inability to produce or secrete GH.
- **Short-statured individuals** respond well to GH therapy.

GH Overproduction

- Excess GH *before* the closure of long bone epiphyses leads to **gigantism**.
- Excess GH *after* epiphyseal closure results in **acromegaly**, characterized by bone overgrowth and characteristic facial and body changes.

Prolactin Axis

Production and secretion of Prolactin (PRL)

1. Mainly from **mammotropic (lactotropic) cells** of the **anterior pituitary**.
2. **Extrapituitary sources:** lymphocytes, skin fibroblasts, brain, mammary gland, decidua (uterine lining during pregnancy), prostate, adipose tissue.

Main function of Prolactin

- Primary stimulating factor for **lactation in the postpartum period**.
- During pregnancy, the pituitary gland doubles in size, and intense PRL secretion prepares the mammary gland for postpartum lactation.
- Broad spectrum of **other functions** – involvement in immune system regulation, reproductive function, and metabolism.

Key Prolactin signaling pathways

1. **Jak/STAT** signaling pathway (see Fig. 4-11);
 - PRL binding induces **dimerization** of the PRL receptor and **phosphorylation of Jak2**.
 - This leads to **phosphorylation** of STAT5, STAT3, and STAT1.
 - Activated STAT5 **dimerizes and translocates into the nucleus**, where it binds to promoters of target genes.
2. PRL can also activate **mitogen-activated protein kinase (MAPK)** (see Fig. 4-6);
3. Activation of the **phosphatidylinositol-3-kinase (PI3K)/Akt** pathway (see Fig. 6-2).

Regulation of Prolactin secretion

Inhibitors – primarily dopamine, as well as norepinephrine, GABA, serotonin, histamine, somatostatin, cholecystokinin, orexin-A, nitric oxide, etc.

Activators – thyrotropin-releasing hormone (TRH), ghrelin, vasoactive intestinal polypeptide (VIP), angiotensin II, estradiol, endogenous opioids, serotonin, vasopressin, neurotensin, bombesin, substance P, oxytocin, neuropeptide Y, galanin, calcitonin.

6.1.2 Neurohypophysis Hormones (Posterior Pituitary)

Vasopressin and Oxytocin

They are **nonapeptides** (9 amino acids).

They are synthesized as **prohormones** in the **hypothalamic nuclei** (supraoptic nucleus (SON) and paraventricular nucleus (PVN)) and mature as they **travel down the axon**, transported in a **complex with carrier proteins (neurophysins)**.

The **axon terminals** end in the **posterior pituitary**, where the hormones are directly secreted into systemic circulation via axo-vasal synapses.

Effects of Vasopressin (AVP, also called antidiuretic hormone - ADH)

Previously, vasopressin was thought to have only **two effects**:

1. **Vasoconstriction**
2. **Antidiuresis**

It is now known that vasopressin exhibits **diverse effects** through its **three receptors (V1a, V1b, and V2)** which are G-protein-coupled receptors (GPCRs) on various cell types.

1. **V1a Receptors (Gq/PLC-IP₃-Ca²⁺ pathway)**

- Found in vascular smooth muscle, liver, platelets, and brain.
- Vasoconstriction (↑ blood pressure).

- Glycogenolysis in the liver.
 - Behavioral effects in the brain (aggression, anxiety, stress response).
2. **V1b (V₃) Receptors (Gq/PLC-IP₃-Ca²⁺ pathway)**
 - Found in the anterior pituitary, pancreas, and brain.
 - Stimulates ACTH release, enhancing the hypothalamic-pituitary-adrenal (HPA) axis response to stress.
 - Involved in glucose homeostasis and insulin secretion modulation.
 3. **V2 Receptors (Gs/cAMP-PKA pathway)**
 - Found in the renal collecting ducts and endothelial cells.
 - Increases aquaporin-2 (AQP2) expression, promoting water reabsorption and antidiuresis.
 - Stimulates release of von Willebrand factor (vWF) and factor VIII, affecting blood coagulation.

Stimuli for Oxytocin (OXT) secretion

1. **Childbirth**
 - **Labor-related events** are a **classical physiological stimulus** for OXT secretion into the bloodstream, as OXT is a functional part of **Ferguson's reflex**.
2. **Lactation**
 - Another **classical stimulus** for OXT secretion is **suckling during lactation**, as OXT is a key player in the **milk ejection reflex**.
 - Circulating OXT binds to **OXTR** (oxytocin receptors) on myoepithelial cells surrounding the **mammary ducts**, causing their **contraction**.
 - This leads to **increased intraluminal (intramammary) pressure** and milk expulsion from the alveolar lumen.
3. **Sexual interactions and stimulation**
 - In both men and women, **sexual activity** and **stimulation** are associated with increased OXT secretion.
 - OXT levels **rise in romantic couples** and remain elevated for **up to six months**, which may explain the **increased** OXT activity during early romantic bonding. Higher oxytocin levels correlate with greater partner attachment, trust, and relationship satisfaction.
4. **Stress-related stimuli**
 - **Exposure to various stressors** also activates OXT secretion into the bloodstream.
 - Such stressors include **physical exercises, social-emotional stress, and osmotic stress**.

Unlike vasopressin, OXT is known to have only **one receptor**, which belongs to the **rhodopsin-type (Class I) G-protein (Gaq11)** family.

It activates **phospholipase C**, leading to **IP₃** and **diacylglycerol** release, which in turn causes **Ca²⁺** release from intracellular stores and activation of **protein kinase C**.

7. Hormones regulating water-salt metabolism

Renin-Angiotensin-Aldosterone System (RAAS) (Fig. 7-1)

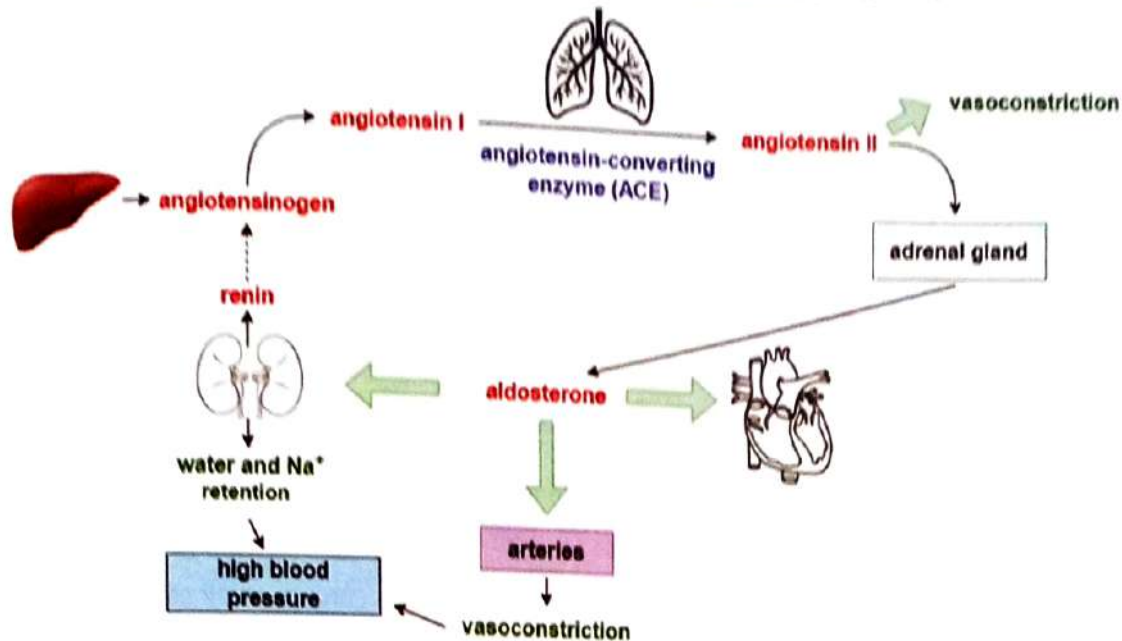


Fig. 7-1 RAAS

RAAS is responsible for the **regulation of blood pressure**.

1. When blood pressure **decreases**, the enzyme **renin** is released from the **juxtaglomerular cells of the kidneys**.
2. The function of **renin** is to **cleave** a 10-amino acid peptide (**decapeptide**) from the **N-terminal of angiotensinogen**, called **angiotensin I**.
3. In the **lungs**, **angiotensin I** is cleaved by the action of **angiotensin-converting enzyme (ACE)**, producing the active hormone, **angiotensin II**. ACE removes 2 amino acids from the C-terminal of angiotensin I.

Angiotensin II is also known as **hypertensin** and **angiotonin**. It is one of the most potent naturally occurring **vasoconstrictors**.

Angiotensin II stimulates the **zona glomerulosa cells** by binding to a **membrane receptor** associated with **phospholipase C**. **PKC** is activated, and **intracellular Ca²⁺** levels increase. These events lead to an increase in **desmolase (P450scc)** activity and enhanced **aldosterone** production.

Effects of Angiotensin II

1. The **vasoconstrictive action** of **angiotensin II** is primarily exerted on the **arterioles**, leading to an **increase in systolic and diastolic blood pressure**.
2. **Angiotensin II** also acts on the **brain**, resulting in **increased blood pressure**, **enhanced secretion of vasopressin and ACTH**, and **increased water intake**.
3. It induces the synthesis and secretion of **aldosterone** from the **adrenal cortex**.
4. **Angiotensin II** affects the contractility of mesangial cells in the **kidneys**, leading to a **reduction in the glomerular filtration rate**.
5. An additional effect of **angiotensin II** is the **potentiation of norepinephrine release**.

Two groups of **antihypertensive drugs** act specifically on the **renin-angiotensin system**.

1. **ACE Inhibitors**

- Block **ACE**, thereby reducing the synthesis of **angiotensin II** and lowering **aldosterone secretion**.
- This leads to **peripheral vasodilation** and a **decrease in blood pressure**.
- ACE inhibition also results in **reduced inactivation of bradykinin**, which explains the occurrence of **dry cough** as an **adverse drug reaction**.

2. **Sartans**

- Selectively block **angiotensin (AT1) receptors**, preventing **angiotensin II** from **exerting its effects**, leading to **lowered blood pressure**.
- They **do not increase bradykinin levels** and therefore **do not cause dry cough**.
- Sartans are an alternative to ACE inhibitors.

Natriuretic peptides (NUP)

Natriuresis refers to the **enhanced excretion of sodium**. This occurs in certain diseases as well as due to the action of **diuretic medications**. At least **three natriuretic hormones** have been identified.

Atrial Natriuretic Peptide (ANP)

ANP is the **first** natriuretic hormone discovered in the **heart**. This hormone is secreted by **cardiac muscle** in response to **increased sodium chloride intake** and **expansion of extracellular fluid volume**. The active ANP is a 28-amino acid peptide, containing a 17-amino acid ring formed by an interchain disulfide bridge.

Brain Natriuretic Peptide (BNP)

BNP was first isolated from the **brain** of a pig, but it was later identified in the human heart and blood. BNP is encoded by a **different gene**. In humans, BNP is **predominantly synthesized** and secreted by **cardiomyocytes** in the **ventricles** of the heart, particularly in response to **ventricular stretch** and **pressure overload** (e.g., heart failure).

C-Type Natriuretic Peptide (CNP)

CNP is **predominantly expressed** in the **brain**. It plays a role in *neuronal function, vascular homeostasis, and bone growth*.

CNP is found in **endothelial cells, fibroblasts, and cardiac tissues**. It is produced in the heart but in much lower amounts compared to ANP and BNP. Unlike ANP and BNP, CNP does not have a major role in **natriuresis** or **diuresis**; instead, it modulates *vascular tone and endothelial function*.

Actions of NUP (Fig. 7-2):

1. NUP act on the **kidneys** by **increasing the glomerular filtration rate (GFR)**, leading to **natriuresis** (enhanced sodium excretion) and **diuresis** (increased fluid excretion). These **renal effects of NUP** are **potassium-sparing**, unlike most diuretic drugs.
2. They **reduce renin** release, thereby **lowering angiotensin II** and **aldosterone** levels, which further promotes **natriuresis** and **diuresis**.

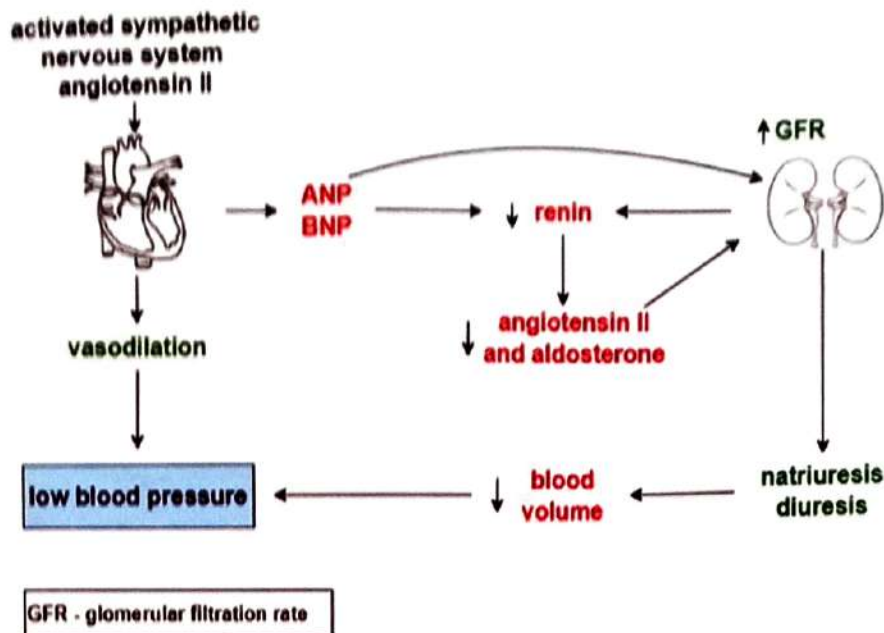


Fig. 7-2 Actions of NUP

Taken together, these actions of NUP **decrease blood volume, arterial pressure, central venous pressure, pulmonary pressure, and heart rate.** They serve as a **counter-regulatory system to RAAS.**

ANP, BNP, and CNP activate **guanylate cyclase**, leading to the formation of **cGMP**.

Parathyroid hormone (PTH)

PTH **increases Ca^{2+} levels in the blood.** The role of PTH is to regulate Ca^{2+} concentration in extracellular fluids. It is synthesized in the chief cells of the parathyroid glands in response to low Ca^{2+} levels.

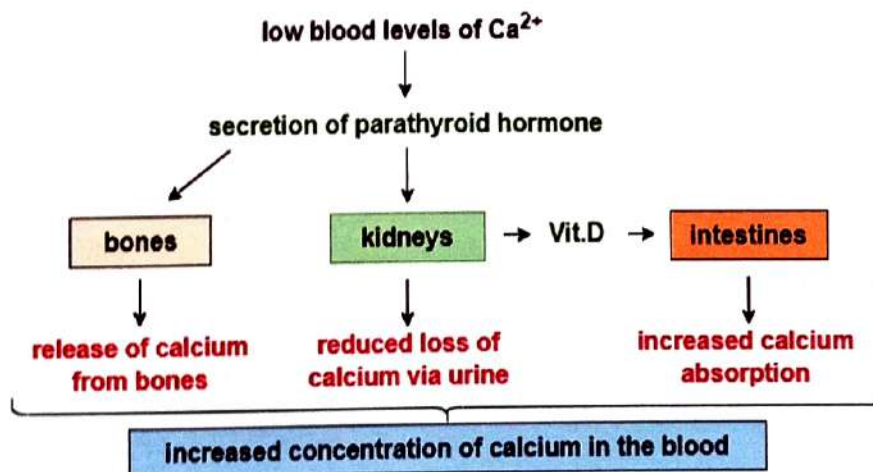


Fig. 7-3 Effects of PTH

Physiological effects of PTH (Fig. 7-3):

1. PTH induces **bone resorption** by stimulating osteoclast activity, leading to an increase in plasma Ca^{2+} and phosphate levels.
2. PTH **reduces renal excretion** of Ca^{2+} by stimulating its reabsorption; simultaneously, it decreases phosphate reabsorption, leading to increased phosphate excretion.

3. PTH acts on the intestines by stimulating vitamin D₃ synthesis in the kidneys (**activating the key regulatory enzyme 1 α -hydroxylase**), which is responsible for Ca²⁺ absorption in the intestines.

Parathyroid hormone-related peptide (PTHrP)

PTHrP and PTH are synthesized from **different genes**. Unlike PTH, which is expressed in the parathyroid glands, PTHrP is widely expressed in various tissues such as the *heart, skin, bone marrow, and fetal liver*. It acts in a paracrine, autocrine, and intracrine manner.

PTHrP binds to the PTH receptor (PTHr1), a **G-protein-coupled receptor (GPCR)**. This activates **protein kinase A (PKA)** or **protein kinase C (PKC)** signaling pathways. It is involved in **skeletal malignancies and bone metabolism**, causing hypercalcemia similar to PTH.

Calcitonin (CT) and Calcitonin gene-related peptide (CGRP)

From a **single gene**, calcitonin is synthesized in the **thyroid gland and pulmonary neuroendocrine cells**, while CGRP is synthesized in the **brain and blood vessels**. These peptides arise through **alternative splicing**. They share common sequences but also have specific differences.

CGRP acts locally and plays a role in **pain perception** (e.g., migraine).

Procalcitonin functions as an **acute-phase protein**.

Parathyroid hormone (PTH), PTHrP, and vitamin D are the primary regulators of calcium homeostasis, while calcitonin has a minor role in calcium regulation in humans.



8. Pancreatic hormones

Insulin

Synthesis of Insulin – see Fig. 2-2

When mature granules are secreted into circulation via exocytosis, both **insulin** and an **equimolar amount of C-peptide** are released.

Secretion of Insulin from β -Cell – see Fig. 3-22 from Medical Biochemistry Part I

Secretory vesicles transfer **proinsulin** from the granular endoplasmic reticulum to the Golgi apparatus, whose aqueous environment, rich in **zinc and calcium**, favors the formation of soluble zinc-containing proinsulin hexamers. Pancreatic β -cells release 0.25–1.5 units of insulin per hour during fasting (or basal) conditions. This level prevents uncontrolled hydrolysis of TAG and limits GNG, thereby maintaining normal fasting blood glucose levels.

Glucose is the primary stimulus for insulin secretion, but other factors also influence it: *amino acids (arginine), fatty acids, acetylcholine, pituitary adenylate cyclase-activating polypeptide (PACAP), gastric inhibitory peptide (GIP), glucagon-like peptide-1 (GLP-1)*, and several other agonists, acting together in combination.

Insulin and physical exercise activate GLUT4 in adipose, muscle tissue, and heart.

The **phosphatidylinositol-3-kinase (PI3K)** pathway is responsible for **GLUT4** activation. In response to a stimulus such as glucose, insulin secretion is characteristically biphasic, with an *initial rapid phase* of insulin secretion followed by a *second phase of less intense* but more sustained hormone release.

Effect of insulin on carbohydrate metabolism

Insulin acts at several stages of carbohydrate metabolism:

1. Facilitated **diffusion of glucose into adipose and muscle cells through GLUT4 translocation (PI3K pathway)**.
2. **Glycogen synthesis** is increased, and glycogen breakdown is reduced, respectively, through **dephosphorylation of glycogen synthase and glycogen phosphorylase kinase**.
3. **Glycolysis** is stimulated, and gluconeogenesis is inhibited through **dephosphorylation of pyruvate kinase and PFK-2**.
4. By signaling intracellular energy abundance, insulin enhances the irreversible conversion of **pyruvate to acetyl-CoA** by activating the **pyruvate dehydrogenase complex**. Acetyl-CoA can then be directly oxidized through the **Krebs cycle** or used for **fatty acid synthesis**.

Effect of Insulin on lipid metabolism

1. **Stimulates fatty acid synthesis** in adipose tissue, liver, and lactating mammary gland, along with the **formation and storage of TAG** in adipose tissue and liver. Fatty acid synthesis is increased through **dephosphorylation of acetyl-CoA carboxylase**, while fatty acid oxidation is suppressed by inhibiting *acyl-carnitine translocase*.
2. **TAG synthesis is stimulated** through *esterification of glycerol-3-phosphate*, while TAG breakdown is suppressed by **dephosphorylation of hormone-sensitive lipase**.

3. **Cholesterol synthesis** is increased by **dephosphorylation of HMG-CoA reductase**, while cholesterol ester breakdown is inhibited through dephosphorylation of *cholesterol esterase*.
4. **Phospholipid metabolism** is also activated by insulin.

Effect of insulin on protein metabolism

Insulin activates protein synthesis in various tissues:

1. It affects the **transcription of specific tRNA**, **translation of mRNA** for proteins in ribosomes, as well as mRNA for *glucokinase, pyruvate kinase, fatty acid synthase, glucose-6-phosphate dehydrogenase and albumin in the liver, pyruvate carboxylase in adipose tissue, casein in the mammary gland, amylase in the pancreas, etc.*
2. Insulin action **reduces** mRNA for hepatic enzymes such as *carbamoyl-phosphate synthetase*, a key enzyme in the urea cycle.
3. The effects on **translation** are widespread and influenced by both insulin itself and various growth factors, e.g., IGF-1. It stimulates **growth, DNA replication, and cell proliferation**.

Insulin transduction system

Insulin mediates its actions by binding to the **insulin receptor**. It has two isoforms and is a **heterotetramer** consisting of **two α and two β** glycoprotein subunits linked by **disulfide bonds**. It is located on the **cell membrane** and has **tyrosine kinase activity**.

Insulin binds to the **extracellular α -subunit**, leading to a conformational change that allows **ATP** to bind to the **intracellular β -subunit**. **ATP phosphorylates the β -subunit**, activating its **tyrosine kinase activity**. The autophosphorylated β -subunits of the receptor **phosphorylate** proteins called **insulin receptor substrates (IRS)**.

Phosphorylated IRS specifically binds to conserved domains of various proteins, called **SH2 domains**. SH2 domains are directly responsible for signal transduction and the manifestation of insulin's effects. Such proteins with SH2 domains include the regulatory subunit of phosphatidylinositol-3-kinase, GRB2, and others.

Key insulin signaling pathways (Fig. 8-1)

IRS proteins (IRS1, IRS2, IRS3, IRS4, etc.) are the main substrates for the insulin receptor. They possess a **PH domain** (pleckstrin homology), which allows binding to **PIP2** (Phosphatidylinositol 4,5-bisphosphate), **PIP3** (Phosphatidylinositol 3,4,5-trisphosphate), and others. Once IRS is phosphorylated, it attaches to proteins with SH2 domains, such as **PI3K**, **GRB2** (Growth Factor Receptor-Bound Protein 2), etc.

There are **three** pathways, each with different unique effects:

1. Phosphatidylinositol-3-kinase (PI3K) Pathway

Main function: Involved in glucose metabolism, glycogen and protein synthesis, cell survival, and GLUT4 translocation.

Mechanism:

- Insulin binds to the **insulin receptor**, which is a *tyrosine kinase* receptor.
- This leads to the **autophosphorylation** of the β -subunits of the receptor and activation of **insulin receptor substrate (IRS) proteins**.

- **IRS proteins** recruit and activate **phosphatidylinositol-3-kinase (PI3K)**, which phosphorylates **phosphatidylinositol-4,5-bisphosphate (PIP2)** to **phosphatidylinositol-3,4,5-trisphosphate (PIP3)**.
- PIP3 activates **PDK1** (phosphoinositide-dependent kinase 1), which then phosphorylates and activates **Akt** (Protein Kinase B, PKB).
- Akt mediates several key effects:
 - **Stimulates GLUT4 translocation** to the plasma membrane → **increased glucose uptake** in muscle and adipose tissue.
 - **Activates glycogen synthase** by inhibiting **GSK3** (glycogen synthase kinase 3) → **enhances glycogen synthesis**. (When GSK3 is inhibited, it cannot phosphorylate and inactivate *glycogen synthase*. As a result, *glycogen synthase* remains active and catalyzes the synthesis of glycogen from glucose. Inhibition of GSK3 by insulin is **key** to regulating energy storage in the form of glycogen, particularly in the liver and muscle. In insulin resistance or type 2 diabetes, this mechanism may be impaired, leading to reduced glycogen synthesis and elevated blood glucose levels.)
 - **Inactivates FOXO1**, reducing gluconeogenesis in the liver. (Akt phosphorylates FOXO1, leading to its export from the nucleus to the cytoplasm and subsequent degradation. This reduces the expression of gluconeogenic enzymes (such as PEPCK and G6Pase), thereby suppressing gluconeogenesis in the liver.)

Activates **mTOR** (mammalian target of rapamycin) → **stimulates protein synthesis**.

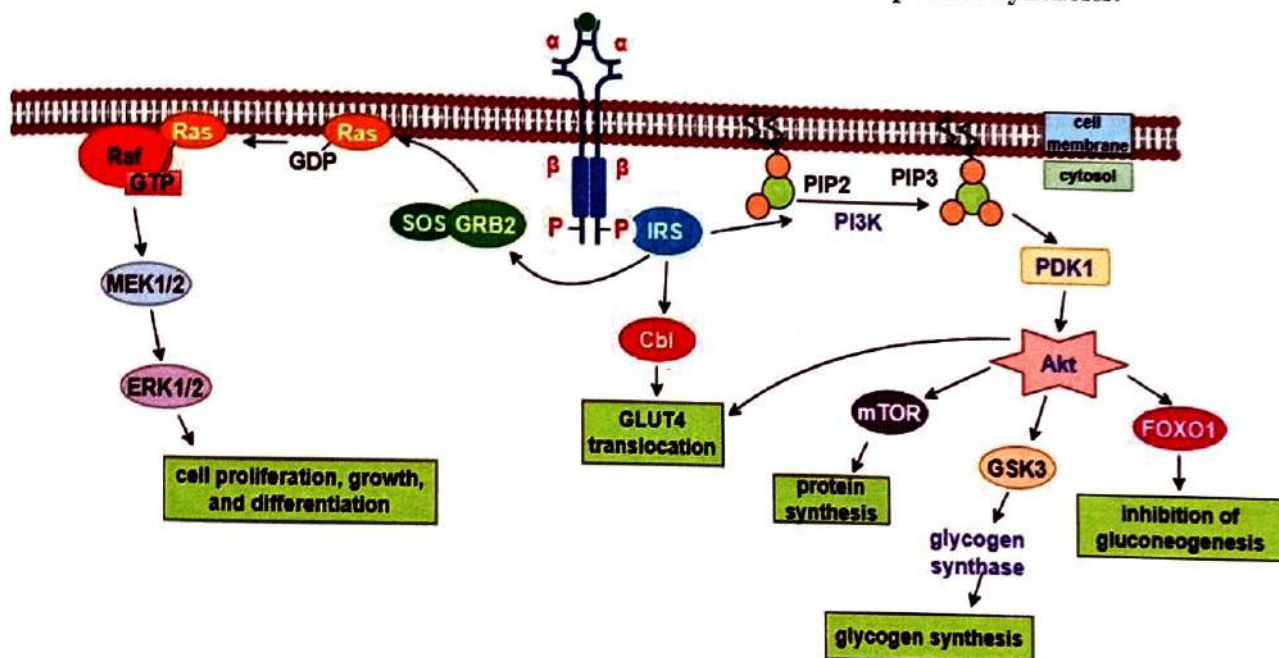


Fig. 8-1 Key insulin signaling pathways

2. MAPK (Mitogen-Activated Protein Kinase) Pathway

Main function: Regulates cell proliferation, growth, and differentiation.

Mechanism:

- After the activation of the insulin receptor, IRS proteins recruit **GRB2** (Growth Factor Receptor-Bound Protein 2) and **SOS** (Son of Sevenless).
- The GRB2/SOS complex activates **Ras**, a small GTPase.

- Ras initiates the **MAP kinase cascade**, activating **Raf**, which in turn activates **MEK**, and MEK activates **ERK1/2** (Extracellular signal-Regulated Kinase 1/2).
- **ERK1/2** enters the nucleus and regulates genes involved in **growth, proliferation, and differentiation** of cells.

3. **Cbl-CAP Pathway (PI3K-independent)**

Main function: Regulates glucose transport through **GLUT4 translocation**, but independently of PI3K.

Mechanism:

- The insulin receptor activates **Cbl** (Casitas B-lineage Lymphoma protein), which binds to **CAP** (Cbl-associated protein).
- This complex associates with specific lipid raft domains in the membrane.

This leads to the activation of **TC10** (a small GTPase), which regulates **GLUT4 translocation** independently of PI3K.

Glucagon

Glucagon is a **polypeptide** composed of 29 amino acids. The hormone is synthesized by the **α -cells of the pancreatic islets of Langerhans** as a much larger **proglucagon** molecule. Similar to insulin, glucagon lacks a plasma transport protein and, like insulin, has a half-life of about 5 minutes. Due to this characteristic, glucagon's primary activity is exerted **in the liver**.

It binds to **membrane receptors** associated with **G-proteins** and **adenylate cyclase**. As a result, **cAMP** levels increase, and **PKA** activity rises, counteracting all the effects described above for insulin's actions in the liver.

Glucagon's effects lead to a significant **increase in circulating glucose**, as it stimulates hepatic **glycogenolysis** and **gluconeogenesis**.

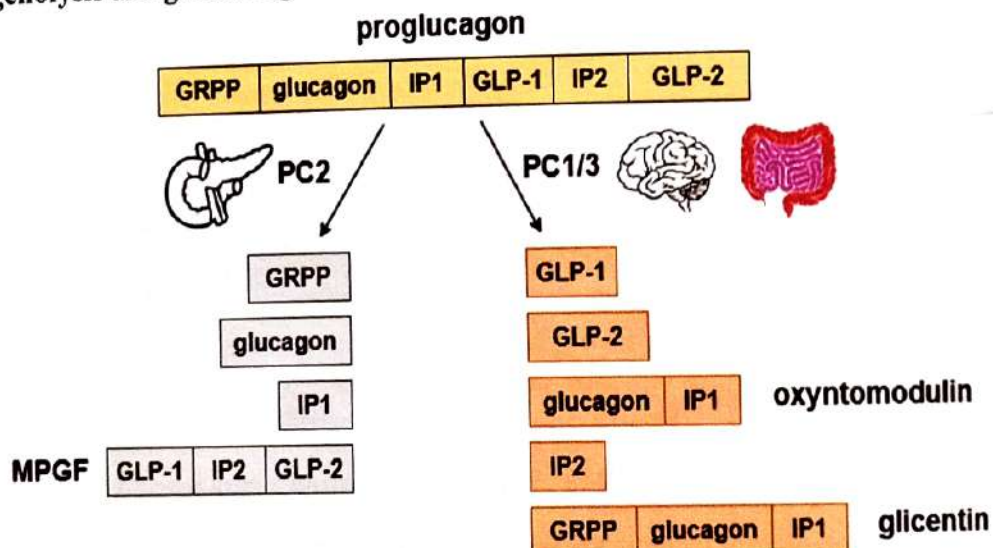


Fig. 8-2 Synthesis of glucagon

Synthesis of Glucagon (Fig. 8-2)

In the pancreas, proglucagon is processed into **glucagon**, **glicentin-related pancreatic polypeptide** (GRPP), **intervening peptide-1** (IP1), and **major proglucagon fragment** (MPGF) by *prohormone convertase-2* (PC2). In the intestines and brain, proglucagon is

cleaved by *prohormone convertase-1/3* (PC1/3) into glucagon-like peptide-1 (GLP-1), glucagon-like peptide-2 (GLP-2), oxyntomodulin, intervening peptide-2 (IP2), and glicentin.

Somatostatin

Somatostatin from **pancreatic δ -cells** is **structurally identical** to **hypothalamic somatostatin**. Somatostatin exists in two biologically active forms: **SS-14** (14 amino acids) and **SS-28** (28 amino acids). The 14-amino acid form (SS-14) is *predominant* in pancreatic δ -cells and the hypothalamus.

Somatostatin **inhibits growth hormone (GH) secretion in the nervous system**. In the hypothalamus, somatostatin (also called Growth Hormone-Inhibiting Hormone, GHIH) inhibits GH secretion from the anterior pituitary by *blocking Growth Hormone-Releasing Hormone (GHRH) action*. This leads to systemic effects on metabolism, growth, and insulin sensitivity.

Somatostatin **in the pancreas acts as a paracrine inhibitor of other pancreatic hormones**. In the pancreas, somatostatin *inhibits insulin and glucagon secretion* by acting locally (paracrine effect). It also inhibits *exocrine pancreatic enzyme secretion*, reducing digestive activity.

Somatostatin secretion responds to blood glucose levels. Somatostatin levels increase not only in response to high blood glucose but also after food intake (*especially protein and fat-rich meals*). Its main role is to prevent excessive hormone secretion, acting as a "*universal inhibitor*" of glucagon, insulin, and digestive processes. It inhibits glucagon secretion when glucose is high, but also suppresses insulin secretion, preventing excessive insulin action.



9. Catecholamines

Synthesis of Catecholamines – see Fig. 5-21 from Medical Biochemistry Part I

The three important catecholamines in humans are **dopamine**, **adrenaline** (epinephrine), and **noradrenaline** (norepinephrine).

DOPA is converted into **melanin** in the melanocytes of the skin, stimulated by **melanocyte-stimulating hormone (MSH)**.

Catecholamines are produced in *adrenergic, noradrenergic, and dopaminergic neurons*.

The neurons of the **substantia nigra** produce dopamine, which reaches the **corpus striatum** via axonal transport. Here, dopamine is **stored** in the granules of the terminals, ready for release into synapses.

In sympathetic ganglion cells, dopamine is oxidized by *dopamine β -hydroxylase* to form **noradrenaline**.

The adrenal medulla is a modified sympathetic ganglion formed from neuroectodermal cells. It is the source of circulating adrenaline and, to a lesser extent, noradrenaline.

Adrenergic Receptors – see Fig. 4-2

Catecholamines act on **adrenergic α - and β -membrane receptors**:

- **α -receptors** are located on cells more sensitive to noradrenaline, less sensitive to adrenaline, and much less sensitive to isoprenaline (a synthetic catecholamine).
 - **$\alpha1$ -Gq-linked receptors** ($\alpha1A$ -, $\alpha1B$ -, and $\alpha1D$) activate **protein kinase C (PKC)**. The $\alpha1$ receptors are activated mainly by **noradrenaline** more than adrenaline. This results in *vasoconstriction, increased blood pressure, and contraction of smooth muscle in the bladder, eye, and blood vessels*.
 - **$\alpha2$ -Gi-linked receptors** ($\alpha2A$ -, $\alpha2B$ -, and $\alpha2C$) inhibit membrane **adenylate cyclase**. This leads to inhibition of noradrenaline release (negative feedback), reduced insulin secretion, by reducing cAMP, suppressing PKA, and decreasing Ca^{2+} influx into beta cells. This serves as a protective mechanism, ensuring glucose is preserved in the blood for energy-demanding organs (brain, heart, muscles) during sympathetic activation (stress, hypoglycemia, physical exertion).
- **β -receptors** ($\beta1$, $\beta2$, $\beta3$) are located on cells most sensitive to **isoprenaline**, less sensitive to adrenaline (and noradrenaline). They are primarily **G α s-linked** and activate **adenylate cyclase**.

Effects of Adrenaline

1. Increases **lipolysis** in adipose tissue, raising **plasma fatty acid levels**.
2. Enhances **gluconeogenesis** in the liver for brain energy supply; stimulates **glycogenolysis** in liver and muscle cells – see Fig. 1-37 from Medical Biochemistry Part I.
3. Moderately **inhibits insulin release** from the pancreas to preserve glucose and shift muscle metabolism toward **fatty acid oxidation**.

This physiological response, characteristic of stress, can be **harmful** to organ function when chronic, particularly affecting **cardiac muscle**. Chronic stress leads to **insulin resistance, altered glucose and lipid metabolism, and mitochondrial dysfunction**.

Stress responses

Noradrenaline and **corticotropin-releasing hormone (CRH)** induce a state of **arousal and aggression**. CRH inhibits **sexual activity and feeding behavior**.

The **sympatho-adrenal system** triggers a "**fight or flight**" response in acute stress. During stress, adrenaline causes **hyperglycemia and ketoacidosis** (it is a diabetogenic hormone).

Chronic stress activates the **HPA axis** → CRH stimulates ACTH release → ACTH stimulates cortisol production in the adrenal cortex via cAMP signaling.

Cortisol has **long-term effects**, including immune suppression and metabolic changes (increased gluconeogenesis, protein catabolism, and lipolysis).

Acute stress leads to **massive adrenaline release** from the adrenal medulla.

Diabetic individuals experiencing **acute hypoglycemia** secrete large amounts of **catecholamines**. Hypoglycemia triggers the release of catecholamines (adrenaline and noradrenaline) via the sympathetic nervous system, leading to: *increased glycogenolysis and gluconeogenesis (to restore glucose levels) and tachycardia, sweating, tremors, and anxiety (hypoglycemic symptoms)*.

In at-risk individuals, catecholamines **dilate bronchioles** (via β_2 -adrenergic receptors) → improves oxygen intake, **inhibit gastrointestinal motility** (via α -adrenergic receptors) → redirects blood flow to muscles, **and improve long-distance vision** ("tunnel vision") by dilating the pupils (mydriasis) and adjusting the lens (cycloplegia).



10. Gastrointestinal hormones

Gastrointestinal hormones (see Table 10-1) are secreted by enteroendocrine cells in the stomach, pancreas, and small intestine, regulating various digestive functions. Many gut peptides, such as secretin, cholecystokinin, and substance P, also act as neurotransmitters and neuromodulators in the central and peripheral nervous systems.

More than 30 genes for peptide hormones are expressed along the digestive tract, making the small intestine the largest endocrine organ in the body.

Characteristics of Gastrointestinal Hormones:

1. They form families based on structural homology and share a common gene origin.
2. Alternative splicing, differential cleavage, and post-translational modifications contribute to hormone diversity, and formation of 30-40 well-characterized GI peptides.
3. These genes are widely expressed outside the intestine, including in neuroendocrine and cancer cells.
4. Different cell types express distinct products from the same gene (cell-specific expression).
5. These peptides can act via multiple pathways:
 - **Endocrine** → released into the bloodstream (e.g., gastrin, secretin, ghrelin).
 - **Paracrine** → local action on nearby cells (e.g., somatostatin, histamine).
 - **Neurotransmitter** → act in the nervous system (e.g., substance P, VIP).
 - **Autocrine** → regulate the cells that produce them (e.g., gastrin in gastric tumors).

Table. 9-1 Gastrointestinal hormones

Hormone	Main localisation	Effect
gastrin	stomach antrum, duodenum	secretion of pepsine and hydrochloric acid
cholecystokinin (CCK)	duodenum, jejunum	secretion of pancreatic amylase
secretin	duodenum, jejunum	secretion of pancreatic bicarbonate
glucose-dependent insulinotropic peptide (GIP)	small intestine	increases glucose-mediated secretion of insulin; inhibits secretion of hydrochloric acid
vasoactive intestinal peptide (VIP)	pancreas	smooth muscle relaxation; stimulates the secretion of pancreatic bicarbonates
motilin	small intestine	increases gallbladder emptying, insulin secretion from the pancreas, gastrointestinal tract motility and hunger
pancreatic polypeptide (PP)	pancreas	inhibits the secretion of bicarbonates and peptides
enkephalin	stomach, duodenum, gallbladder	opiate-like action
substance P	primary afferent neurons of the gastrointestinal tract etc.	activation of immune system cells, increases vascular endothelium permeability (leading to extravasation of plasma) and stimulates chemotaxis of leukocytes
bombesin and bombesin-like peptides	stomach, duodenum	stimulates the release of gastrin and CCK, secretion of pancreatic enzymes, intestinal motor activity, and other processes such as thermoregulation, glucose homeostasis, circadian rhythms and food intake

Incretins

Glucagon-like peptides, particularly **GLP-1** (Glucagon-Like Peptide-1) and **GIP-1** (Glucose-Dependent Insulinotropic Peptide), belong to the incretin class of molecules.

Incretins **stimulate insulin secretion** from the pancreas following food intake.

Glucagon-Like Peptide-1 (GLP-1)

During digestion, **GLP-1** is secreted by enteroendocrine **L-cells**, primarily in the **ileum and colon**.

Main physiological responses of GLP-1:

1. **On β -cells:**
 - Stimulates glucose-dependent insulin secretion (only when glucose is elevated).
 - Suppresses glucagon secretion, but primarily when glucose levels are high (its effect is glucose-dependent).
 - Increases GLUT-2 gene expression (this is mainly regulated by glucose itself).
 - Enhances glucokinase gene expression in the pancreas.
 - Activates β -cell proliferation.
 - Reduces β -cell apoptosis.
2. **On gastric cells:**
 - Inhibits gastric acid secretion.
 - Delays gastric emptying.

The **delayed gastric emptying** enhances satiety, **reducing** food intake and appetite. The effect of **GLP-1 on insulin and glucagon secretion** significantly **lowers postprandial glucose levels**, which is relevant in **diabetes management**.

All effects of GLP-1 are mediated through the **GLP-1 receptor** (GLP-1R), a serpentine receptor (7-transmembrane) coupled to **G-proteins**. Activation of **GLP-1R** increases **cAMP** production and **PKA** activation, with additional **PKA-independent responses**.

Glucose-Dependent Insulinotropic Peptide (GIP-1)

GIP is a **42-amino acid peptide**, synthesized by **enteroendocrine K-cells**, primarily located in the **duodenum and proximal jejunum**.

1. Primary function: Inhibits gastric secretion, hence the old name gastric inhibitory peptide (GIP).
2. Stimulates glucose-dependent insulin secretion.
3. Activates *lipoprotein lipase*.
4. Promotes β -cell proliferation and survival, but does not affect gastric emptying, unlike GLP-1.

The GIP receptor (GIPR) is a G-protein-coupled serpentine receptor, primarily expressed on β -cells. Type 2 diabetes patients exhibit defective responses to GIP.



11. Hormones with intracellular receptors

General characteristics of nuclear receptors (NRs):

- NRs function as **ligand-dependent transcription factors** that bind to the promoters of specific **DNA sequences**, acting as "intracellular sensors" and regulating *lipid and glucose metabolism*.
- They bind hormones such as **steroid hormones, melatonin, T3, etc.**, and **metabolites** such as **lipids, oxysterols, heme, and bile acids**. They serve as sensors for **redox balance and nutrient levels** in the cell.
- NRs share a similar **domain organization** (**ligand-binding domain, DNA-binding domain**), which is crucial for amplifying the signaling of hormones and metabolites through specifically targeted genes.
- NRs are most strongly expressed in **metabolically active tissues** (liver, muscles, white and brown adipose tissue), some of which exhibit a **24-hour circadian periodicity of expression**.
- The binding of specific ligands to the appropriate receptor induces ligand-induced **conformational changes** in NRs, **NR dimerization, translocation** to the nucleus, **interaction** with target gene promoter elements, release/recruitment of **corepressors or coactivators**, chromatin remodeling, and ultimately interaction with **RNA polymerase II** to initiate transcription.

Hormones with intracellular receptors

These include **steroid hormones, calcitriol, retinoic acid** (a derivative of vitamin A), and **thyroid hormones: T3 and T4**. All of them are **hydrophobic** and easily **pass through the plasma membrane**. In the **cytoplasm or nucleus**, they bind to a **specific receptor**. The **hormone-receptor complex** serves as the **intracellular signal** (the **second messenger**). It binds to a **specific regulatory DNA sequence** called **HRE** (hormone response element). This affects **gene expression** (activation or inhibition), usually the **transcription of specific genes** (Fig. 11-1, 11-2).

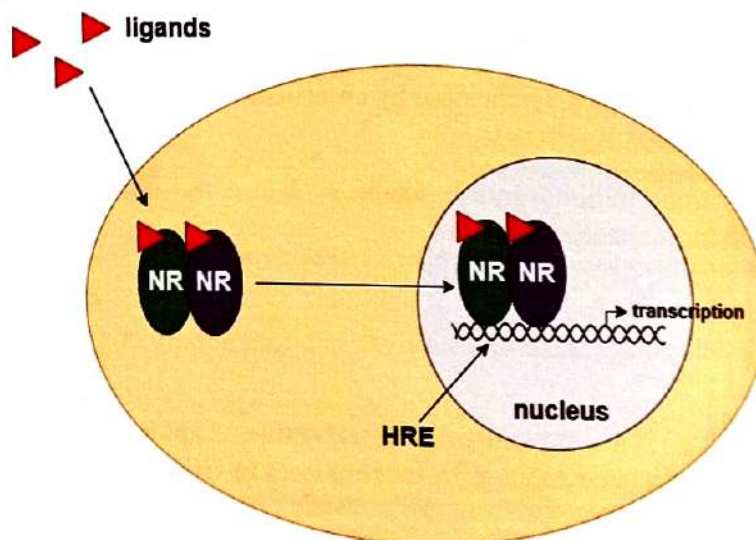


Fig. 11-1 Binding of a specific ligand to an intracellular receptor

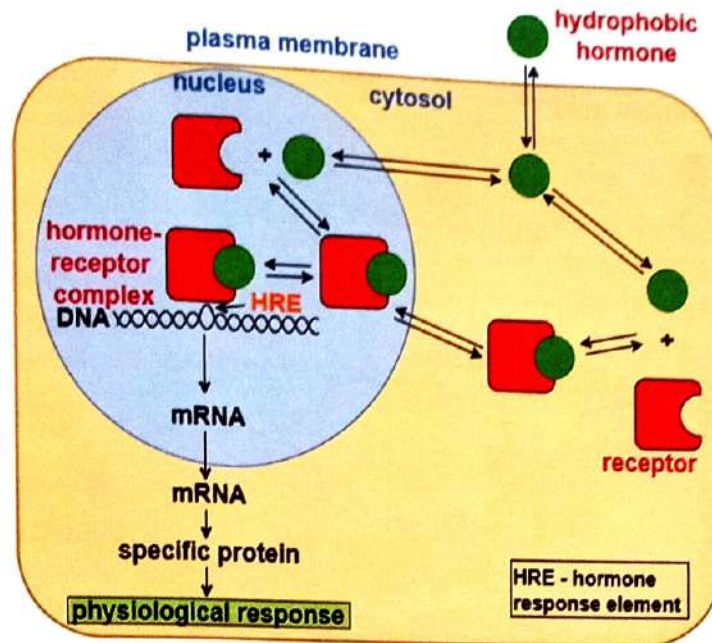


Fig. 11-2 Mechanism of action of hydrophobic hormones

Types of intracellular receptors

Type I Receptors

Steroid receptors (glucocorticoid, mineralocorticoid, androgen, and progesterone receptors), as well as **calcitriol receptors**, are predominantly located in the **cytoplasm** and are classified as type I receptors. They bind to **heat shock proteins** (HSPs) such as hsp90, hsp70, and hsp56. When the hormone binds to the receptors, HSPs are released from the complex, and the receptor forms a **dimer** with another identical and activated receptor (**homodimer**). The dimer then freely enters the nucleus, where it binds to a specific DNA sequence called **HRE**.

Type II Receptors

These receptors are usually found in the **nucleus** and are already bound to DNA. They form **heterodimers** (e.g., the **thyroid hormone receptor** and **retinoid X receptor**) or can initiate **transcription** as **monomers** upon **ligand binding**.

Retinoic Acid Receptors (RARs)

Retinoid receptors (for vitamin A and its derivatives) are designated as **RARs** (**retinoic acid receptors**). They exist in at least three subtypes: **RAR α** , **RAR β** , and **RAR γ** . There is another family of nuclear receptors called **retinoid X receptors (RXRs)**, which represent a second class of retinoid-responsive transcription factors. RXRs **enhance** the DNA-binding activity of RARs, of thyroid hormone receptors, and others. There are three distinct **RXRs** (**α** , **β** , and **γ**). The primary difference between RARs and RXRs is that the former have the highest affinity for **trans-retinoic acid**, whereas the latter have high affinity for **9-cis-retinoic acid**.

Metabolism of Retinoic Acid

1. **Retinol** (vitamin A) is synthesized into **retinal** via **alcohol dehydrogenases (ADHs)** or through **short-chain dehydrogenases/reductases (RDHs/SDRs)**.
2. **Retinal** is oxidized to **retinoic acid (RA)** via **retinal aldehyde dehydrogenases (RALDHs)**. **Retinol** is transported by **cytosolic retinol-binding proteins (CRBPs)**, while **retinoic acid** is bound by other proteins— **cytosolic retinoic acid-binding proteins (CRABPs)**. It is metabolized in the liver (Fig. 11-3).

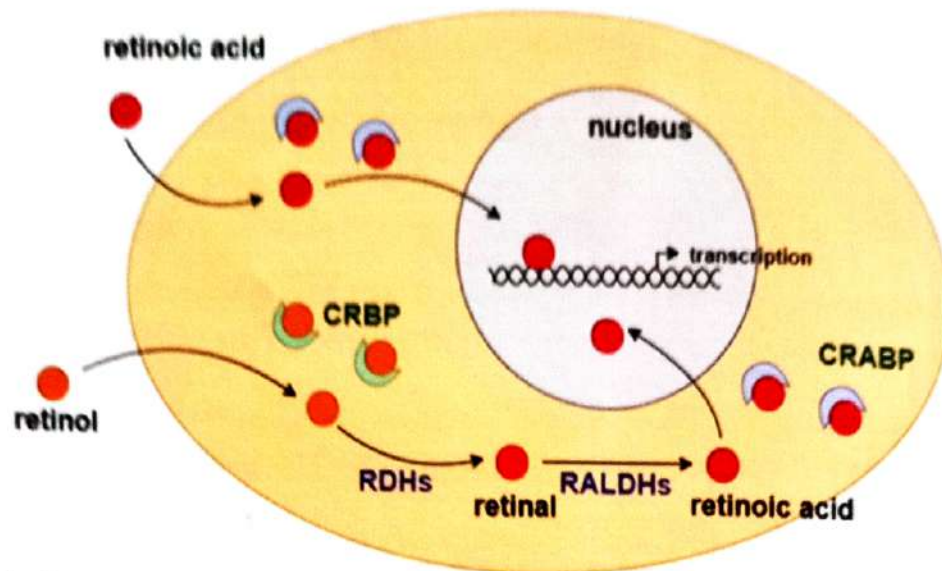


Fig. 11-3 Mechanism of action of retinoic acid

Vitamin A (Biological role)

The role of retinoic acid (RA) as a hormone: after being taken up by cells, it binds to CRABP and is transported to the **nucleus**. There, it binds to **specific nuclear receptors** for RA and participates in the **regulation of gene expression**. The specific nuclear receptors for RA are **RARs (retinoic acid receptors)**, which function as **heterodimers with RXRs (retinoid X receptors)** - RAR/RXR (Fig. 11-4).

In the **absence of the ligand (RA)**, the RAR/RXR heterodimer is bound to DNA with **corepressors**. This complex induces **transcriptional repression** through **histone deacetylation**. Ligand (RA) binding induces **conformational changes** and **coactivator** recruitment, leading to **histone acetylation** and activation of **transcription**.

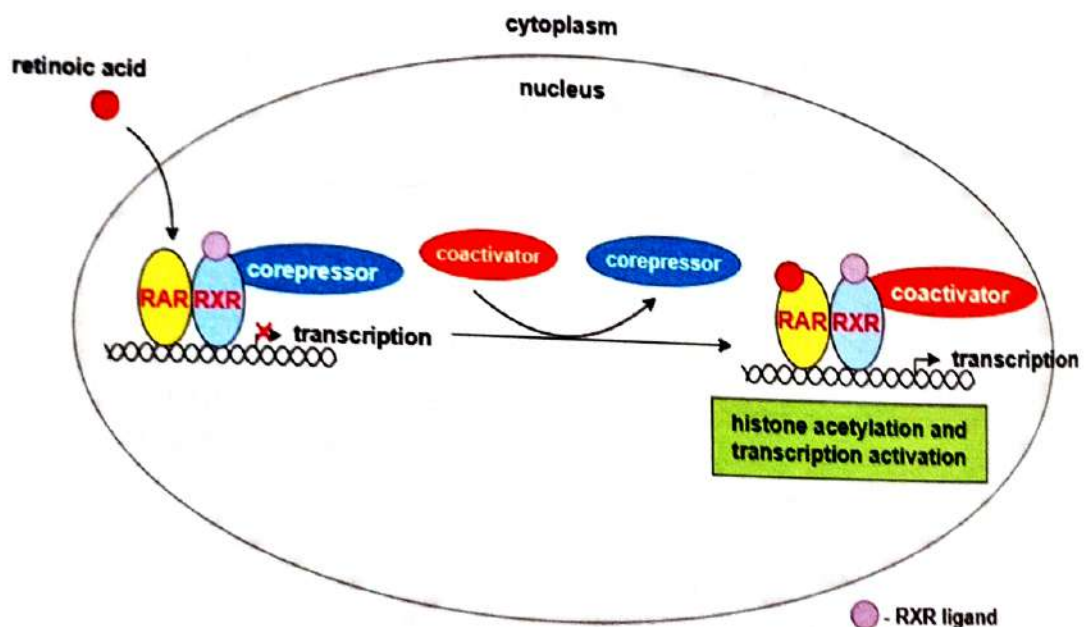


Fig. 11-4 Effect of retinoic acid on transcription

Thyroid Hormone (TH) Receptors

Genomic effects of TH – via Nuclear Receptors

T₃ enters the cell through a transporter or is synthesized locally by cytoplasmic **deiodinases**. In the nucleus, T₃ binds to its receptor **THR**, which is associated with **RXR** (Retinoid X Receptor). The **THR/RXR heterodimer** binds to **TRE** (thyroid hormone response element) to regulate **gene transcription** (Fig. 11-5).

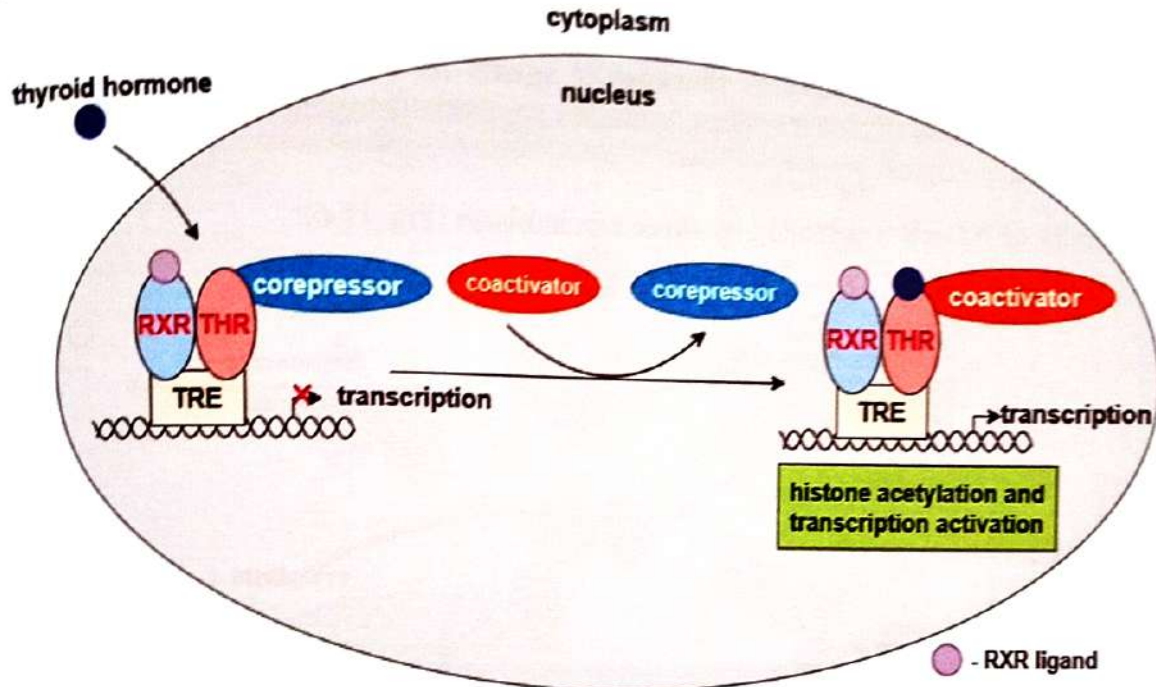


Fig. 11-5 Effect of thyroid hormones on transcription

In the absence of a ligand, **THR** (type II receptor) is bound to a **corepressor** protein. The binding of the ligand to **THR** leads to the dissociation of the **corepressor** and the recruitment of a **coactivator** protein, which, in turn, attracts other proteins such as **RNA polymerase**, responsible for **gene expression** and **DNA transcription**.

Nongenomic effects of TH – see Fig. 16-2

TH can also exert **rapid, nongenomic effects** on **membrane receptors**. **Integrin $\alpha\beta 3$** is a **membrane receptor** for TH in **blood vessels** and the **heart**, as well as in several types of **cancer cells**. The **signaling pathway** involves **PI3K** activation and **protein biosynthesis** via **Akt/PKB** and **mTOR**. Additionally, the activity of the **Na⁺/K⁺ ATPase pump** on the membrane is enhanced.

NF- κ B pathway

The **transcription factor NF- κ B** is a **heterodimeric complex** composed of two subunits, **p50** and **p65**. Normally, **NF- κ B** is retained in the **cytoplasm** in a transcriptionally inactive form by its **inhibitor I κ B**.

Extracellular stimuli, such as **pro-inflammatory cytokines**, **reactive oxygen species**, **viruses**, **bacteria**, and **mitogens**, activate the **I κ B kinase complex (IKK)**, which has a **heterohexameric structure**, consisting of **$\alpha 2$, $\beta 2$, and $\gamma 2$** subunits.

IKK phosphorylates two serine residues in IκB, marking it for **ubiquitination** and subsequent **proteasomal degradation**. Once **IκB** is degraded, **free NF-κB** translocates to the **nucleus**, where it binds to **gene promoters** and activates the **transcription** of genes involved in the **inflammatory response**.

The **transcriptional regulation** by **NF-κB** is mediated by multiple **coactivators**, such as **cAMP response element-binding protein (CREB)**.

Glucocorticoid hormones and NF-κB inhibition

Glucocorticoid hormones serve as **therapeutic agents** for the treatment of **inflammatory diseases** and **immune disorders**. These activities are partially explained by the **inhibition** of **NF-κB** and its biological effects.

The **activity** of **NF-κB** is inhibited by **three mechanisms** (Fig. 11-6):

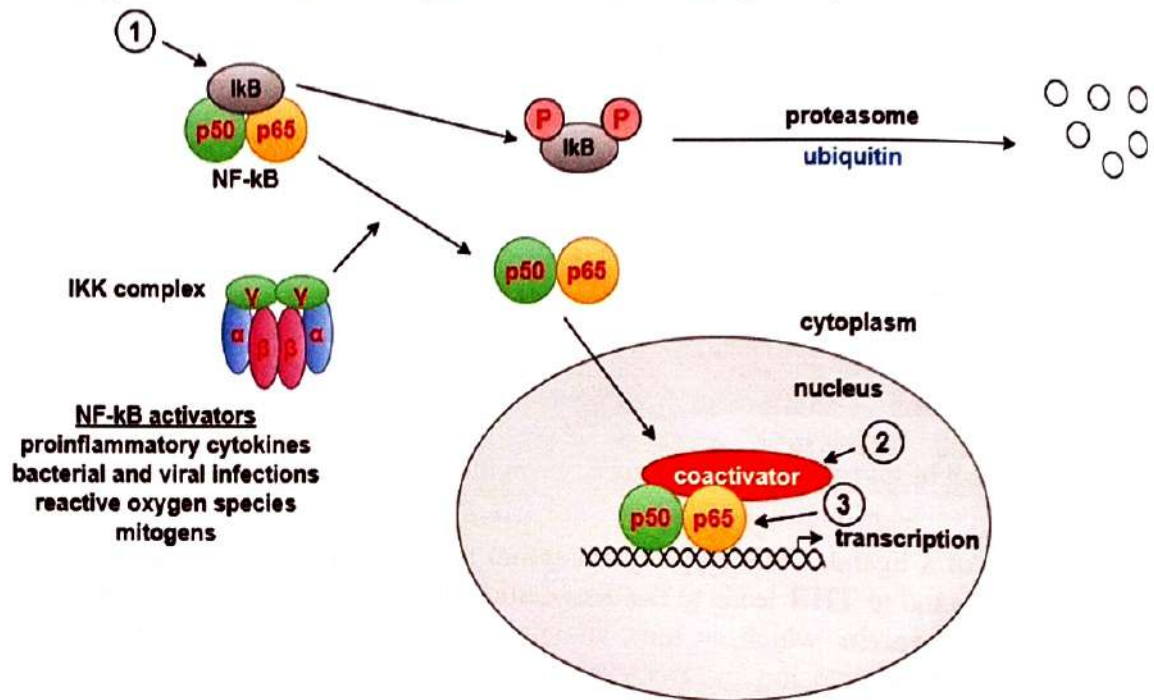


Fig. 11-6 Inhibition of the NF-κB pathway by glucocorticoids

1. **Glucocorticoids** increase **mRNA** for **IκB**, leading to **enhanced retention** of **NF-κB** in the **cytoplasm**.
2. The **glucocorticoid receptor** competes with **NF-κB** for binding to **coactivators**.
3. The **glucocorticoid receptor** directly binds to the **p65 subunit** of **NF-κB**, inhibiting its **activation**.

Genomic and nongenomic effects of estradiol (E2) on eNOS expression and activity

Genomic effects involve **classical intracellular estrogen receptors (ERs)**, which, upon binding to **E2**, interact with the **estrogen response element (ERE)** in **DNA**, thereby **enhancing eNOS expression**.

Nongenomic effects involve the binding of **E2** to **GPER (G-protein coupled estrogen receptor)**, leading to the activation of **various transcription factors**, such as **CREB**, which also induces **eNOS expression**.

Intracellular signals of Vitamin D3

Genomic effects of vitamin D3 (Fig. 11-7)

Calcitriol binds to its receptor - **VDR**, which dimerizes, preferably with **RXR**, forming a heterodimer. This complex binds to **VDRE** (vitamin D response elements) in DNA to regulate transcription.

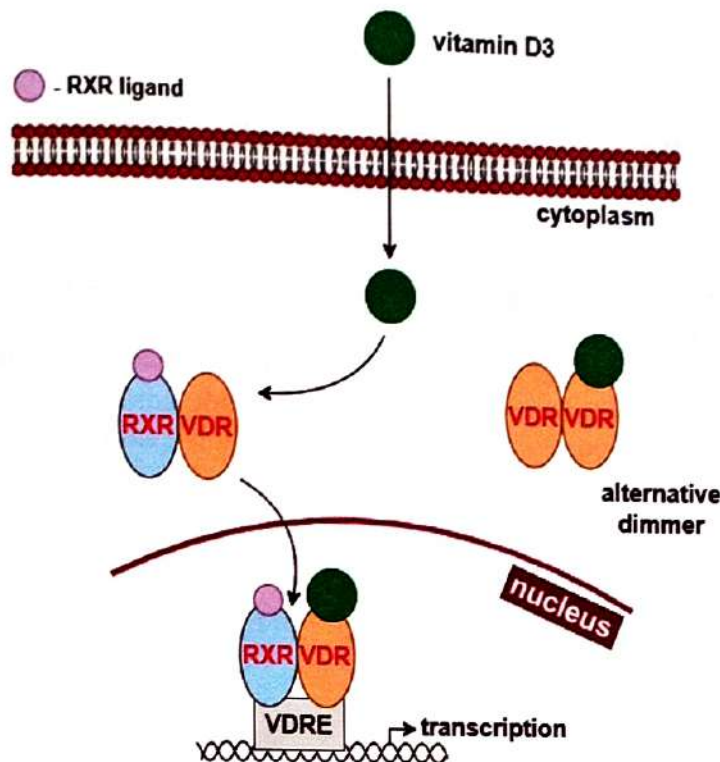


Fig. 11-7 Classical genomic signaling pathway of vitamin D3

Nongenomic effects of vitamin D3

These effects can be mediated through the classical **VDR** or **PDIA3** (protein disulphide isomerase family member 3). Vitamin D3 **rapidly activates protein kinases** such as **CaMKII**, **PKA**, and **PI3K**, facilitating **Ca²⁺ influx** through **L-VGCC** (L-type voltage-gated calcium channel). Intracellular **Ca²⁺** then activates **p38MAPK**, further modulating downstream signaling. Both genomic and nongenomic actions of vitamin D3 affect **calcium metabolism**, as well as the **development, function, and maintenance of the brain and other tissues**, where it can be synthesized and localized.

Additional families of intracellular receptors

Other members of this receptor family (consisting of **48 discovered genes**) bind to various **lipophilic ligands**.

They include:

- **Retinoid X receptors (RXRs)**
- **Liver X receptors (LXRs)**
- **Farnesoid X receptors (FXRs)**
- **Peroxisome proliferator-activated receptors (PPARs)**
- **Pregnane X receptors (PXR)**

Retinoid X Receptors (RXRs)

RXRs belong to the **orphan receptor family**, as their natural ligands were unidentified at the time of discovery. The first identified natural ligand was **9-cis-retinoic acid (9-cis-RA)**.

Other **RXR ligands** include *polyunsaturated fatty acids, such as docosahexaenoic acid (22:6), arachidonic acid (20:4), oleic acid (18:1), and phytanic acid (a derivative of chlorophyll)*.

In the nucleus, **RXR functions as a transcription factor**, binding as a homodimer or heterodimer (with PPARs, LXRs, and FXRs) to **promoter regions of specific genes in DNA**.

There are **three RXR isoforms: RXR α , RXR β , and RXR γ** , each containing multiple isoforms.

Liver X Receptors (LXRs) (Fig. 11-8)

Liver X receptors (LXRs) and retinoid X receptors (RXRs) form **heterodimers** that bind to **LXR response elements (LXRE)** in DNA. The **LXR/RXR complex** recruits corepressors, suppressing gene expression. When activated by **oxysterols or synthetic ligands**, and/or when **RXR is activated by ligands such as 9-cis-retinoic acid**, corepressors are replaced by **coactivators**, activating the **expression of lipid metabolism genes** in response to **cellular cholesterol levels**.

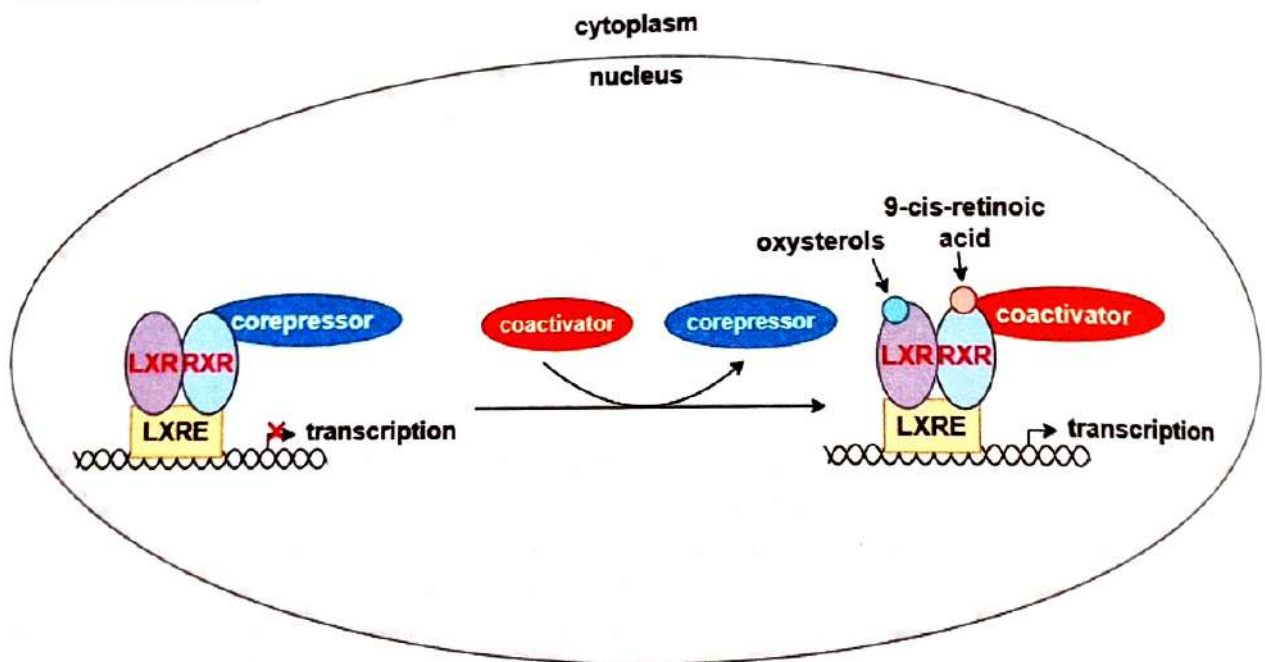


Fig. 11-8 Liver X receptors

- In the liver, LXR stimulates **cholesterol conversion into bile acids**, enhances excretion via cholesterol transporter ABCA1, and has **anti-atherogenic effects**.
- LXR **inhibits cholesterol uptake** in the liver and macrophages by inducing LDL receptor degradation.
- **Suppresses cholesterol and fatty acid biosynthesis** in the liver by inducing SREBP1c (sterol regulatory element-binding protein 1c) and ChREBP (carbohydrate-response element-binding protein).
- In macrophages, LXR **enhances cholesterol transport** to the plasma membrane and incorporation into **ApoA1** (apolipoprotein A1) or **pre- β HDL**.
- In the intestines, LXR stimulates **HDL formation** and **intestinal cholesterol excretion**.

Peroxisome proliferator-activated receptors (PPARs)

- PPARs act as **metabolic sensors**, allowing the body to respond to **environmental changes** via gene expression. There are **three PPAR isoforms**: **PPAR α** , **PPAR β/δ** , and **PPAR γ** .
- **PPAR α** is found in **high-fat catabolism tissues** such as the **liver, heart, kidneys, muscles, and brown adipose tissue**.
- **PPAR β/δ** is **ubiquitously expressed** and enhances **HDL levels**.
- **PPAR γ** is **predominantly in adipose tissue**, primarily involved in **adipocyte differentiation and adipogenesis**.
- PPARs are **key therapeutic targets**, with specific synthetic ligands available for each isoform:
 1. **Fibrates** act as **PPAR α ligands**, used to treat **hyperlipidemia**.
 2. **Thiazolidinediones (TZD)** (Fig. 11-9) are **PPAR γ -specific activators**, increasing **insulin sensitivity** and used for **type 2 diabetes treatment**, enhancing **GLUT4 expression**.

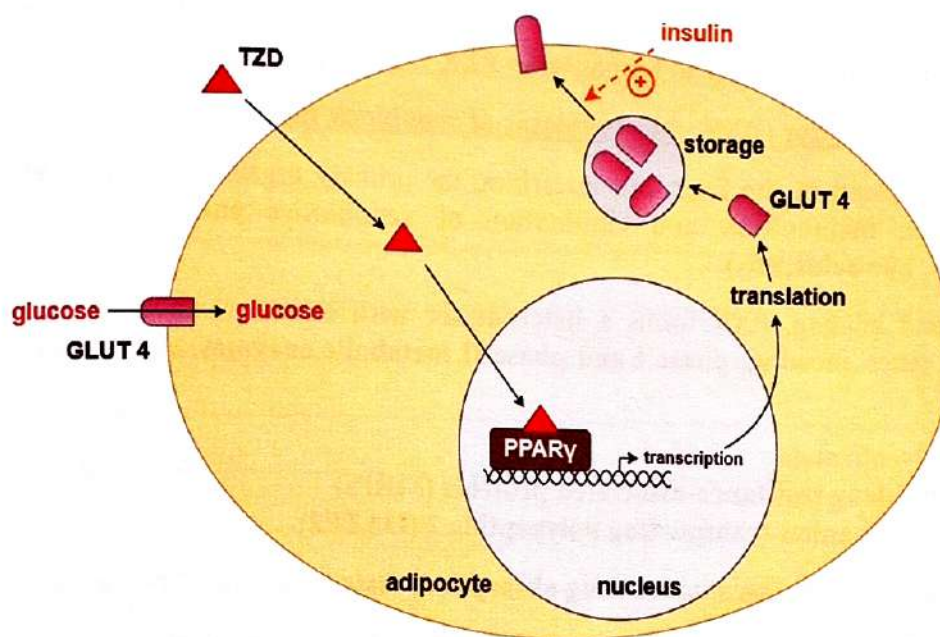


Fig. 11-9 Action of thiazolidinediones (TZDs)

Farnesoid X Receptors (FXRs)

FXR plays a role in maintaining **bile acid homeostasis, cholesterol, and glucose metabolism**.

1. FXR and glucose metabolism

- FXR induces the **KLF11 (Kruppel-like factor 11)** transcription factor, which mediates glucose-induced **insulin gene transcription**.
- FXR also regulates **insulin secretion** via non-genomic mechanisms.
- Activation of FXR in **β -cells** enhances **Akt phosphorylation** and translocation of **GLUT2 to the plasma membrane**, increasing **glucose uptake**.
- FXR also induces **GLUT4 expression** through **FXR elements (FXRE)** in the **GLUT4 promoter**.

2. FXR and bile acid formation (Fig. 11-10)

- FXR is a **nuclear receptor** expressed in the **liver and intestines**.
- **Bile acids** are the **physiological ligands** for FXR.

- *7 α* -hydroxylase (CYP7A1) is the rate-limiting enzyme in bile acid synthesis.
- FXR indirectly suppresses CYP7A1 transcription by inducing small heterodimer partner (SHP) (nuclear receptor co-regulator), regulating bile acid formation via feedback inhibition.

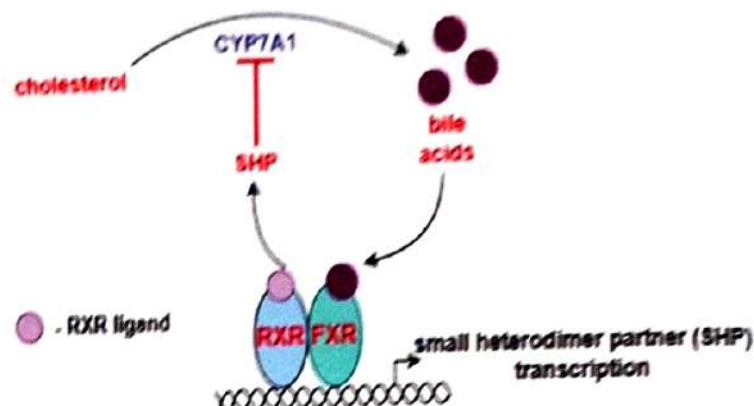


Fig. 11-10 Control of bile acid formation by FXR

Pregnane X Receptor (PXR - key regulator of xenobiotic metabolism)

PXR is expressed in the liver and intestines, the primary organs involved in absorption, distribution, metabolism, and elimination of xenobiotics and endobiotics (steroid hormones, bile acids, etc.).

Upon ligand binding, PXR forms a heterodimer with RXR, which binds to DNA and activates genes encoding phase I and phase II metabolic enzymes, as well as transporters such as:

- P-glycoprotein
- Multidrug resistance-associated proteins (MRPs)
- Organic anion-transporting polypeptide 2 (OATP2)

These transporters are essential for drug absorption, distribution, and excretion.

PXR regulates CYP3A (cytochrome P450 enzyme) gene expression in the liver and intestines. Drug A binds to PXR, inducing CYP3A expression, which accelerates Drug B metabolism, a CYP3A substrate (Fig. 11-11).

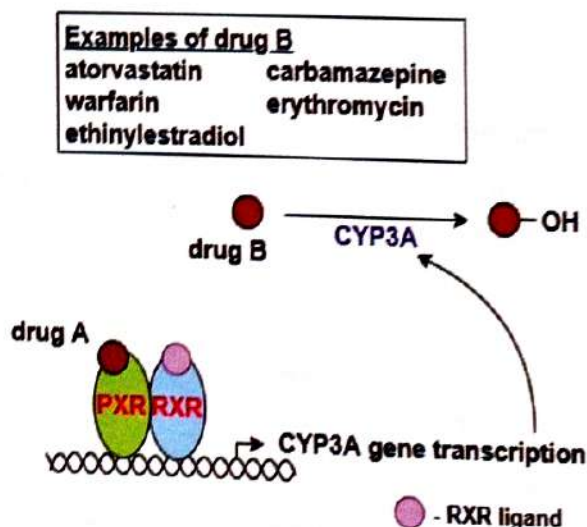


Fig. 11-11 Molecular basis of drug interactions

12. Apoptosis

It is estimated that around **100 billion cells die every day**. These cells must be replaced. **Apoptosis** clears out unnecessary cells through **phagocytosis**, eliminates **old cells**, and/or stimulates **tissue regeneration** in an **adaptive, normal process** that depends on **RNA and DNA synthesis** in the dying cell.

Three main types of **cell death** have been identified in biomedical literature:

1. **Regulated cell death**
2. **Programmed cell death (apoptosis)**
3. **Accidental cell death**

Another classification includes:

Anoikis; autophagic cell death; caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis; cornification; entosis; extrinsic apoptosis by death receptors; mitotic catastrophe; necrosis; necroptosis; netosis; pyroptosis; autolysis; autophagy; autosis; ferroptosis, etc.

Unlike **necrosis**, apoptosis **does not trigger an inflammatory or immune response**.

Apoptosis involves activation of a pathway leading to **cellular self-destruction** through a characteristic process where the **cell becomes more compact**, develops **membrane blebbing**, undergoes **chromatin condensation**, **DNA fragmentation**, and **cellular fragmentation**, forming **apoptotic bodies** that are subsequently **phagocytosed**.

Apoptosis can be initiated by various stimuli, such as **growth factor deprivation**, **glucocorticoid (GC) treatment**, **gamma radiation**, and **activation of specific receptors**. Another pathway is used by the **immune system**, where **cytotoxic T-lymphocytes** attack **target cells**.

Apoptosis can be induced through two **main pathways**:

1. **Extrinsic signals via death receptors and formation of the DISC (death-inducing signaling complex)**
2. **Intrinsic apoptotic programs activation**

Regardless of the pathway, the **final effector phase of apoptosis** involves **activation of effector caspases**, which **degrade cellular substrates**, leading to **cell death**.

Extrinsic apoptotic pathway (receptor-mediated) (Fig. 12-1, 12-2)

The extrinsic apoptotic pathway is initiated by **external signals** when the extracellular environment signals that a cell should undergo programmed death.

Initiation of the Extrinsic Pathway:

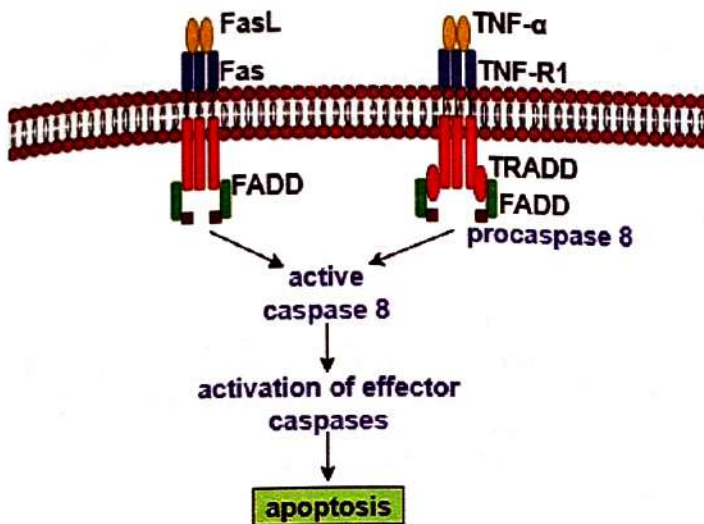
- The **extrinsic apoptotic pathway** is triggered by **external death signals**, such as **FasL and TNF α** , which bind to **death receptors (Fas/CD95, TNF-R1)**.
- These **death receptors trimerize** upon ligand binding, leading to the attraction of **FADD (Fas-Associated Death Domain Protein)** and **TRADD (TNF Receptor-Associated Death Domain Protein)**.

Formation of the DISC complex:

- FADD facilitates the attraction and activation of procaspase-8, forming the DISC (Death-Inducing Signaling Complex).
- Procaspase-8 is cleaved into active caspase-8, which initiates the apoptotic cascade.

Initiation phase of Apoptosis:

- The initiation phase involves caspase-8 activation, leading to:
 - Effector caspase activation (e.g., caspase-3, caspase-7)
 - Mitochondrial changes (in some cases)
 - Further caspase cascade signaling



Execution phase (effector phase) results in:

- Proteolysis of key cellular proteins by caspases
- DNA fragmentation
- Chromatin condensation
- Cell death via apoptotic body formation

Fig. 12-1 Extrinsic pathway of apoptosis

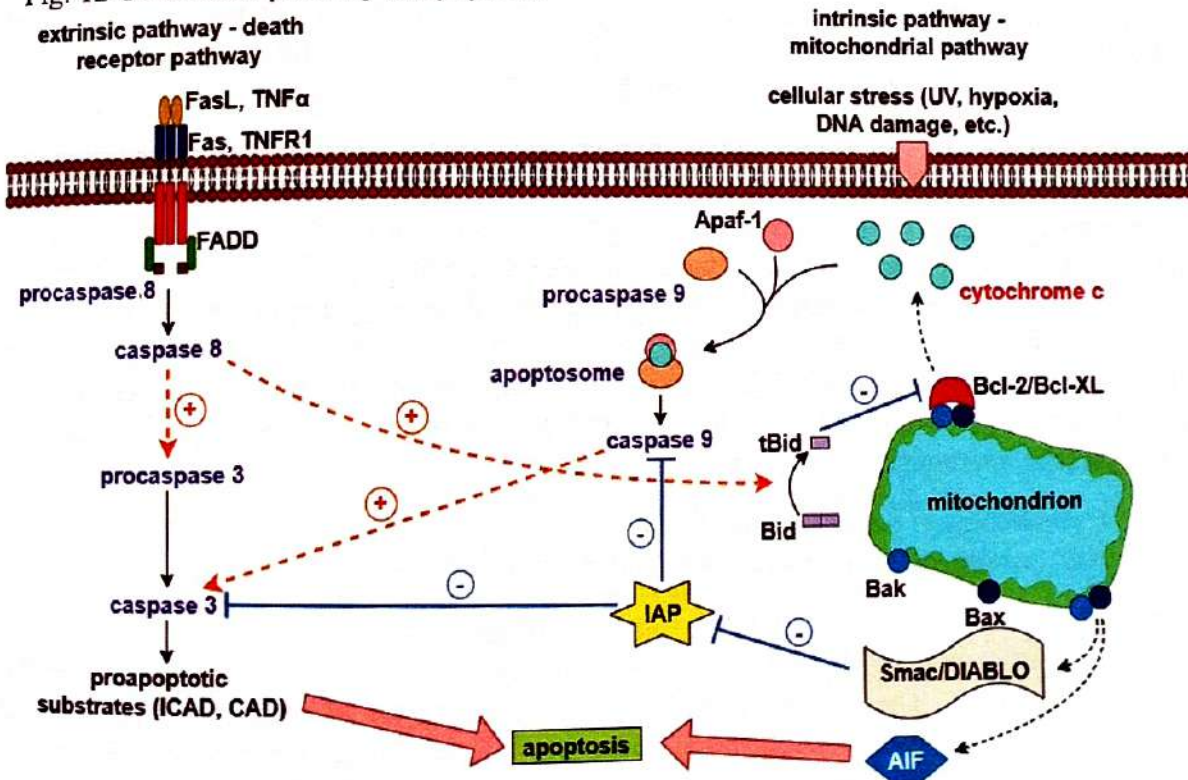


Fig. 12-2 Intrinsic pathway of apoptosis

Intrinsic apoptotic pathway (Fig. 12-2)

The intrinsic apoptotic pathway is initiated in response to **internal cellular damage**.

The **Bcl-2 protein family** forms the **core of the intrinsic apoptotic pathway**. It is an **anti-apoptotic protein** that blocks the mitochondrial pathway of apoptosis by inhibiting **Bax** (Bcl2 Associated X-protein) and **Bak** (Bcl2 Antagonist Killer-1). **Growth factor deprivation, GC exposure, and DNA damage** activate **Bcl-2 proteins** (through **p53** in DNA damage cases, **GC receptors** in GC-related cases, or other signaling proteins in **cytokine deprivation**). **p53** detects cellular stress and plays a key role in activating the intrinsic apoptotic pathway.

Caspase-3 cleaves **ICAD**, the **inhibitor of CAD** (caspase-activated DNase), while **caspase-8** cleaves **Bid** (BH3 interacting-domain death agonist), activating it into **tBid** (Truncated Bid), which **induces dimerization of apoptotic proteins Bax and Bak**. Bax and Bak are the **pore-forming proteins** in the intrinsic apoptotic pathway. This leads to the release of **cytochrome c**, **Smac/DIABLO**, **AIF** (Apoptosis-Inducing Factor), and **EndoG** (endonuclease G) into the cytoplasm, activating caspases and leading to cell death.

Anti-apoptotic proteins Bcl2, Bcl-XL (Bcl2 Related Protein Long Isoform) and other anti-apoptotic Bcl2 family members inhibit **cytochrome c** and **AIF** release by **preventing pore formation** in mitochondria, cause closure.

In the **cytosol**, **cytochrome c** binds **Apaf-1** (apoptosis protease-activating factor), **forming the apoptosome**, which **converts procaspase-9 into caspase-9**, activating **effector caspase-3**.

Smac/DIABLO inhibits **IAPs** (Inhibitors of Apoptosis Proteins), preventing them from blocking **caspase-9** and **caspase-3** activation.

Caspase-independent degradation

AIF plays an **indirect role in chromosome degradation**, as it **activates endonuclease G and CAD (DNase)**, which migrate from mitochondria to the nucleus during **apoptosis**, leading to DNA fragmentation and chromatin condensation.

Role of mitochondria in apoptosis

The increase in cytosolic Ca^{2+} levels is due to the activation of receptors associated with ion channels, such as the glutamate neurotransmitter receptor, which can induce a transient change in mitochondrial membrane permeability (**mitochondrial membrane permeability - MMP**). The change in MMP is the **first rate-determining step** in the overall apoptotic pathway. The alteration of the inner mitochondrial membrane leads to the **opening of pores**, loss of transmembrane potential, and the **release** of two groups of pro-apoptotic proteins from the mitochondrial intermembrane space into the cytoplasm:

1. **Cytochrome c, Smac/DIABLO, and**
2. **AIF (apoptosis-inducing factor), EndoG, and CAD.**

The change in MMP is also related to the formation of toxic **reactive oxygen species (ROS)** in the mitochondria, which play a role in the degradation phase of apoptosis (e.g., changes in the plasma membrane). These ROS cause widespread **oxidative damage** to mitochondrial components. Mitochondrial DNA impairment disrupts oxidative phosphorylation, which contributes to various human diseases.

Caspase cascade

Caspases are a class of **cysteine proteases** (cysteine-dependent aspartate-specific proteases), many of which are involved in apoptosis. Caspases transmit the apoptotic signal in a proteolytic cascade, where some caspases have proteolytic activity on other caspases (procaspases), activating them and leading to the destruction of cellular targets, resulting in cell death.

- **Initiator caspases** include **caspase 8** and **caspase 9**.
 - **Caspase 8** is the initial caspase characteristic of the extrinsic apoptotic pathway. It is activated in response to death domain-containing receptors, where caspase 8 binds to **FADD** (an adaptor protein).
 - The **mitochondrial stress pathway** begins with the release of **cytochrome c** from the mitochondria, which interacts with **Apaf-1**, causing self-cleavage and activation of **caspase 9**.
- **Effector caspases** – **caspases 3, 6, and 7** – are downstream of the activating caspases and act to degrade various cellular targets.
- **Granzyme B** and **perforin** are proteins released by cytotoxic T cells, which induce apoptosis in target cells by forming transmembrane pores and initiating apoptosis through the cleavage of effector caspases, such as **caspase 3**.

Fas signaling pathway (Fig. 12-1, 12-2)

- **Fas (CD95/APO-1)** is a **death receptor** belonging to the **TNF receptor superfamily**.
- **Fas Ligand (FasL)** is part of the **TNF superfamily** and binds **Fas**, triggering apoptosis.

Immune homeostasis function:

- **Fas/FasL-mediated apoptosis** is **critical for immune regulation**, especially in removing autoreactive lymphocytes to prevent autoimmunity and limit tissue damage.

Mechanism of Fas/FasL apoptosis:

- **FasL binding induces Fas receptor trimerization** in the target cell membrane.
- This triggers the **attraction of FADD** via **death domain (DD) interactions**.
- **FADD then attracts procaspase-8**, leading to the **formation of the DISC** (Death-Inducing Signaling Complex).
- **Procaspase-8 is activated into caspase-8**, which **initiates the caspase cascade** by activating **caspase-3**, ultimately causing **cell death**.

Inhibitors of apoptosis

Apoptosis can be effectively blocked at various stages by different proteins:

- The inhibitory protein **FLIP** (inhibits caspase 8);
- **Bcl-2**;
- **Cytokine response modifier A (CrmA)** (inhibits caspases 1, 6, and 8), or
- **IAP** (inhibitor of apoptosis proteins) (causes polyubiquitination of **RIPK1** – RIP kinase).

Phosphatidylinositol-3-Kinase (PI3K) in cell survival signaling (Fig. 12-3)

Activation of the **PI3K/Akt** pathway has two distinct outcomes:

1. Activation of the **NF- κ B** pathway (see Fig. 10-6) and cell survival, and
2. Involvement of **14-3-3** proteins in the inhibition of apoptosis.

Role of 14-3-3 proteins:

- 14-3-3 proteins **modulate apoptosis** by **sequestering phosphorylated forms** of certain pro- and anti-apoptotic proteins in the cytoplasm, **preventing their interaction with mitochondrial components**.
- This **inhibits apoptotic signaling**.

Phosphorylation of Bad:

- **Bad** (Bcl-2-associated agonist of cell death) is a **pro-apoptotic** protein that, in its **dephosphorylated state**, binds to **anti-apoptotic Bcl-2/Bcl-XL** in the mitochondria.
- This **neutralizes Bcl-2/Bcl-XL**, leading to **apoptosis**.
- When **Bad is phosphorylated** (by kinases such as Akt or PKA), it **binds to 14-3-3** proteins in the cytoplasm.
- This prevents its interaction with **Bcl-2/Bcl-XL**, thereby **inhibiting apoptosis**.

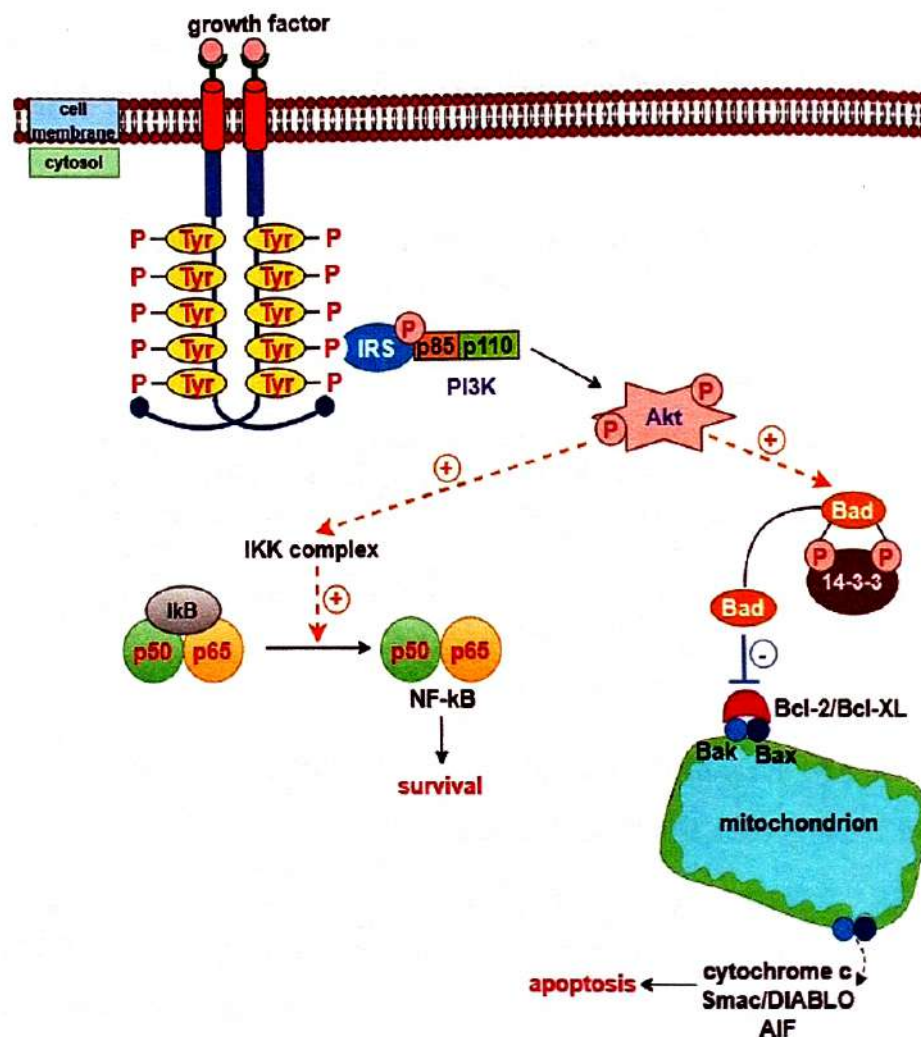


Fig. 12-3 Role of PI3K in cell survival signaling

p53 signaling pathway

The tumor suppressor protein **p53** possesses a specific DNA-binding sequence, directly interacts with various cellular and viral proteins, and induces cell cycle arrest in response to DNA damage.

In response to signals from various genotoxic stresses, such as UV radiation, DNA damage, hypoxia, etc., **p53** is expressed and undergoes post-translational modification, leading to its accumulation in the nucleus.

Through **p53-dependent classical pathways**, genomic stability is maintained via:

1. **Apoptosis;**
2. **DNA repair;**
3. **Temporary cell cycle arrest, or**
4. **Permanent arrest (Fig. 12-4).**

For example, in response to γ -radiation, **p53** activates the transcription of the **p21CIP1** protein, which binds to and inhibits cyclin-dependent kinases (CDKs), leading to hypophosphorylation of the retinoblastoma protein (pRb). This prevents the release of the transcription factor **E2F** and blocks the cell cycle. Some of the cellular effects of **p53** can be blocked by dysregulation of **c-Myc**, **Bcl-2**, or **E2F**. The activity of **p53** is autoregulated by Mdm2. The binding of **Mdm2** to **p53** directs **p53** for ubiquitination and degradation, inhibiting **p53-induced cell cycle arrest and apoptosis**.

The non-classical effects of **p53** include pathways of **autophagy**, **necrosis**, **necroptosis**, and **ferroptosis**.

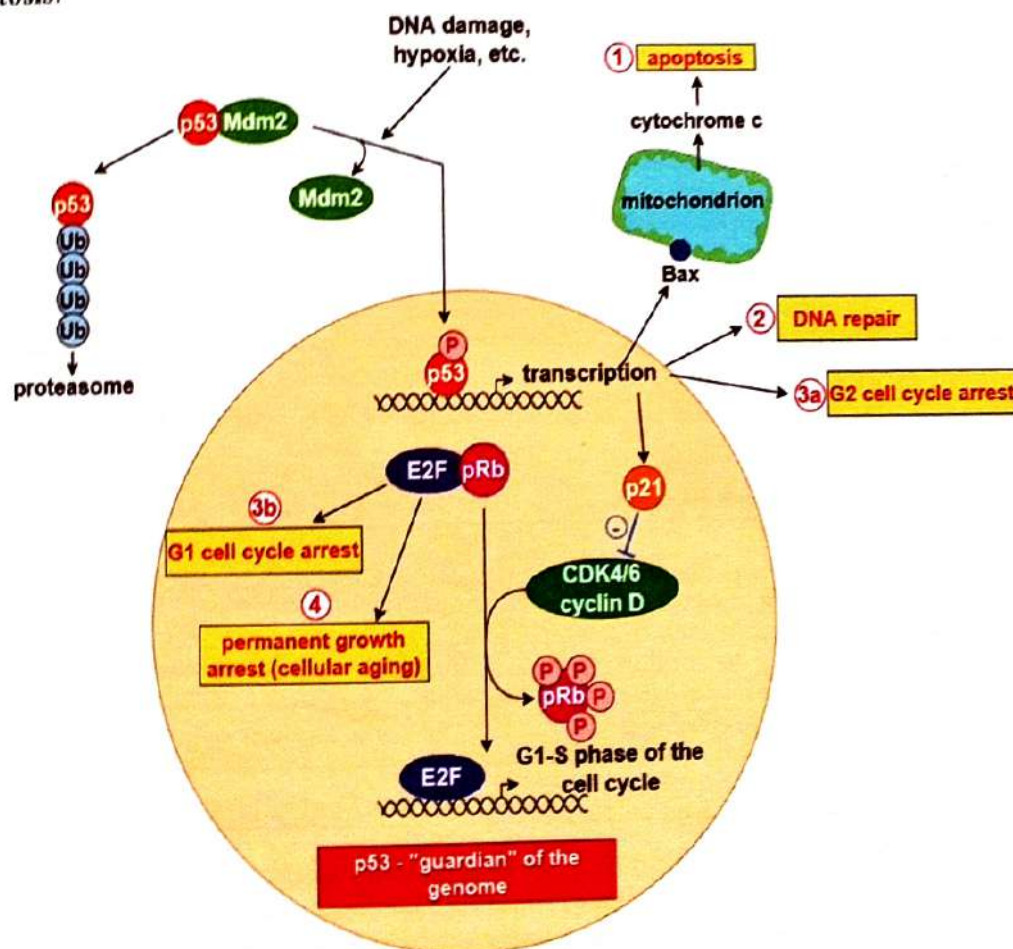


Fig. 12-4 p53 Signaling Pathway

13. Mechanisms of oncogenesis

Characteristics of tumor cells

Tumor cells are characterized by:

1. **Morphological changes** – Tumor cells are more *rounded* compared to normal cells.
2. **Loss of contact inhibition** – Normal cells form a *monolayer*, while malignant cells grow in *multiple layers*.
3. **Reduced or uncontrolled growth regulation.**
4. **Invasiveness** – The ability to *penetrate* and *damage* surrounding tissues.
5. **Metastasis** – The ability to *spread* through the *blood* and *lymphatic system* to other organs, forming *secondary tumor sites*.
6. **Reduced dependency on growth factors** – Tumor cells often *secrete* their own *growth factors*, allowing *autonomous proliferation*. *Benign tumor cells* also show *reduced growth control* but *do not* invade local tissue or metastasize.
7. **Alterations in cytoskeletal structure** – Changes in *actin filaments*.
8. **Genetic abnormalities** – Genes controlling *growth* and *interactions with normal cells* exhibit *abnormal structure* or *deregulation*.
9. **Decreased apoptosis.**
10. **Aneuploidy** – Deviations from the *normal chromosome number*.
11. **Overproduction of enzymes, proteins, and hormones** – Tumor markers can be detected in *plasma* or *serum*.

Table 13-1 Some tumor markers

Marker	Localization
carcinoembryonic antigen (CEA)	large intestine, lungs, mammary gland, pancreas
α -fetoprotein (AFP)	liver, germ cells
human chorionic gonadotropin (hCG)	trophoblast, germ cells
calcitonin (CT)	thyroid gland (medullary carcinoma)
prostatic acid phosphatase (PAP)	prostate
chromogranin A (CgA)	neuroendocrine tumors
estrogen receptor (ER)/progesterone receptor (PR)	breast cancer
gastrin	gastrin-producing tumor (gastrinoma)
prostate-specific antigen (PSA)	prostate
thyroglobulin	thyroid gland

Application of tumor markers (Table 13-1)

1. **Screening** – Used for **early detection** in **asymptomatic patients**. No **universal tumor marker** has been identified to detect all types of cancer.
2. **Differentiation between benign and malignant tumors (diagnosis).**
3. **Prediction of treatment effectiveness and monitoring for recurrence.**

4. Selection of therapy and **prognosis** of tumor progression.
5. Staging of the disease (**classification**).
6. Tumor localization – After injection of **radioactive material**, the tumor site is **identified**.
7. Cytotoxic therapy – Targeting **tumor marker-expressing cells**.

Causes of cancer

Cancer-causing agents fall into **three major categories**:

1. Radiation energy

- **Ultraviolet (UV) rays, X-rays, and γ -radiation** damage DNA.
- **UV rays** cause the formation of **thymine dimers**.
- **X-rays and γ -radiation** induce **DNA strand breaks** and **cross-links**, leading to **mutagenic and carcinogenic effects**.
- Radiation also generates **reactive oxygen species (ROS)**, which cause further DNA and macromolecule damage.

2. Chemical carcinogens

- **80% of tumors** are linked to chemical exposure.
- Environmental factors include **occupational toxins, aflatoxins (mold byproducts), benzene, ethylene oxide, tobacco smoke (benzo[a]pyrene), and certain drugs**.
- Chemical carcinogens are classified as **organic or inorganic** and exert their effects through two mechanisms:
 - **Genotoxic (DNA-reactive) effects** – Direct **chemical interaction** with DNA.
 - **Epigenetic (non-genotoxic) effects** – Indirect modification of DNA, such as **oxidative stress**, leading to **DNA damage and uncontrolled cell proliferation**.

The human diet contains **both carcinogenic and anti-carcinogenic components**, influencing **cancer development**.

3. Viruses, bacteria, and parasites

Direct carcinogens and procarcinogens

The mechanism of action of **organic carcinogens** is the best-studied. They are classified into:

- **Direct carcinogens** – Act **immediately** on target molecules.
- **Procarcinogens** – Require **metabolic transformation** to exhibit **tumorigenic activity**.
The process of converting **procarcinogens** into **active carcinogens** is called **metabolic activation**.

The **intermediate products** formed during this process are called **intermediate carcinogens**, while the final product, which reacts directly with a **cellular component** (e.g., DNA), is referred to as the **ultimate carcinogen**. The sequence is:

Procarcinogen → Intermediate Carcinogen A → Intermediate Carcinogen B → Ultimate Carcinogen

Ultimate carcinogens are often **electrophilic** molecules (**electron-deficient**) and thus have a **high affinity** for **nucleophilic (electron-rich)** groups in DNA, RNA, and proteins.

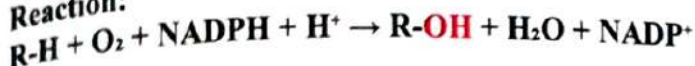
Activation of procarcinogens

The metabolism (biotransformation) of procarcinogens and other xenobiotics occurs in two phases (see Chapter 20):

Phase I of Xenobiotic Metabolism (Hydroxylation Phase) – Occurs in the endoplasmic reticulum

- Monooxygenases catalyze the nonspecific hydroxylation of various xenobiotics and procarcinogens.
- This process requires oxygen, NADPH as a reductant, and cytochrome P450 (which facilitates electron transport).
- The resulting product becomes more polar, making it more suitable for further excretion.

Reaction:



- Hydroxylation reactions can result in the formation of either active or inactive forms of a drug.

Phase II of Xenobiotic Metabolism (Conjugation Phase)

- Hydroxylated compounds undergo conjugation with molecules such as glucuronic acid, acetate, and glutathione.
- This process increases their polarity (hydrophilicity), facilitating their elimination from the body (mainly via urine).
- In some cases, conjugation enhances the biological or chemical activity of a molecule, as seen in the formation of ultimate carcinogens.

The enzymes responsible for conjugation reactions (glucuronyl transferase, sulfotransferase, acetyltransferase, and glutathione transferase) are primarily located in the cytosol, though some are found in the endoplasmic reticulum.

Stages of chemical carcinogenesis

I. Initiation

- A rapid and irreversible process.
- Involves permanent modification of DNA, leading to one or more mutations.

II. Promotion

- A slow process that occurs over months or years.
- Various compounds, including phenobarbital and saccharin, can act as promoters.
- Protein kinase C is believed to function as a membrane receptor for some promoters (e.g., phorbol esters from croton oil).
- This leads to increased phosphorylation of certain membrane proteins, altering transport and other cellular functions.

Example of Promotion and Initiation:

- Repeated application of benzo[a]pyrene to a specific area of mouse skin does not cause skin cancer.
- However, if croton oil is applied to the same site after benzo[a]pyrene treatment, multiple tumors develop.
- In this case, benzo[a]pyrene serves as the initiator, while croton oil acts as the promoter.

Oncogenes and proto-oncogenes

Oncogenes are DNA segments (genes) whose function leads to **tumor transformation**. The first **oncogenes** were found in **oncogenic viruses** and identified as factors responsible for **transformation**.

This DNA sequence, homologous to viral oncogenes, is called a **proto-oncogene**. Proto-oncogene products participate in **normal differentiation and cellular proliferation**, while **oncogene products** contribute to **tumor growth**. An **oncogene** present in a tumor cell is referred to as "**c-onc**" (e.g., **c-ras**), whereas **proto-oncogenes** in normal cells are labeled "**c-onc-proto-oncogene**". The **viral oncogene** is labeled "**v-onc**" (e.g., **v-ras**), and the corresponding **proto-oncogene** as "**v-onc-proto-oncogene**".

Mechanisms of proto-oncogene to oncogene conversion

1. **Insertion of a promoter** – introducing a *viral promoter* near the gene activates it.
2. **Insertion of an enhancer** – inserting a *viral enhancer* near the gene activates it.
3. **Chromosomal translocation** – a *chromosome fragment detaches and joins another*.
4. **Gene amplification** – *abnormal gene multiplication*, leading to multiple copies. This occurs in *oncogenes* and *drug resistance genes* in tumors.
5. **Point mutation** – *classic mutation in the ras oncogene*.
6. **Gene rearrangement** – *complex genomic rearrangements*, often seen in *lymphomas*.

Mechanisms of oncogene action

There are at least **three mechanisms** by which **oncogenes stimulate tumor growth**:

1. Oncogenes **impact key intracellular processes** controlling **tumor growth**. For example:
 - **src oncogene** – a tyrosine protein kinase
 - **ras oncogene product** – stimulates adenylate cyclase activation
 - **myc oncogene product** – stimulates DNA-binding protein formation

In each case, oncogenes influence **mitosis**.

2. Oncogene products **imitate growth factors**, initiating **cellular transformation**.
3. Oncogene products **mimic receptor stimulation**, leading to **continuous activation**. Some **oncogene products** are **mutated receptors**, constantly **active**.

Growth Factors (GFs) (Fig. 4-7)

GFs influence various cells (e.g., **blood cells, neurons, mesenchymal, epithelial cells**). Their effect is **mitogenic**, but some inhibit growth (**TGF- β**).

GFs act through **three pathways**:

- **Endocrine**
- **Paracrine**
- **Autocrine**

Like peptide hormones, GFs **transmit signals across the cell membrane** via **membrane receptors** with **tyrosine kinase activity**.

K-Ras (Fig. 13-1)

The mutant **K-Ras**, which is always in the "ON" position, activates signaling pathways, many of which lead to **uncontrolled growth and proliferation of cancer cells**. This makes **K-Ras** an **attractive target for treatment**. However, the mutated K-Ras is a very difficult protein to target with anti-cancer drugs.

In patients with colorectal carcinoma, additional antibody therapy targeting the EGF receptor (EGFR) (such as cetuximab or panitumumab) can be used, but only in patients with the "wild-type" (non-mutated) K-Ras gene. The reason for this is that EGFR and other related receptor proteins rely on K-Ras for transmitting proliferation signals.

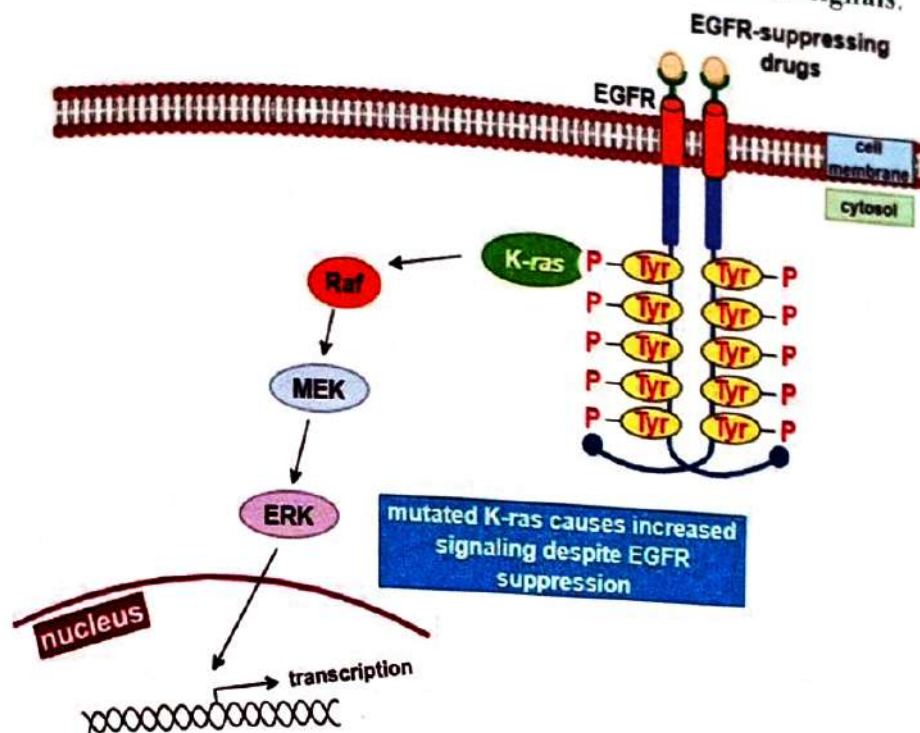


Fig. 13-1 K - RAS signaling pathway

Oncogenic viruses

Oncogenic viruses contain either RNA or DNA.

DNA Oncogenic viruses

1. **Polyomavirus and SV40 virus** – small viruses with a **circular genome**, coding 5-6 proteins. Under certain conditions, they cause **malignant transformation**.
2. **Adenoviruses** – include **Epstein-Barr virus**, associated with **Burkitt's lymphoma** and **nasopharyngeal carcinoma**.
3. **Hepatitis B virus** – linked to **liver cancer**.
4. **Herpesvirus-2** – associated with **cervical cancer**.

RNA Oncogenic viruses

These are **retroviruses** that **synthesize cDNA via reverse transcriptase**, which integrates into the **host genome**. The integrated cDNA is called a **provirus**, and its **oncogene** is a **v-proto-oncogene**.

- Examples: **T-cell leukemia virus type I & II**, **Rous sarcoma virus** is well studied.

Tumor suppressor genes (anti-oncogenes) – proteins inhibiting cell proliferation

Rb is a tumor suppressor gene involved in the development of **retinoblastoma**.

It is called a **tumor suppressor gene** because **mutations** in this gene lead to the development of **retinoblastoma**. The gene responsible for retinoblastoma, **Rb1**, has been isolated and sequenced, and its product, **pRb**, has a molecular mass of 110 kDa and is **expressed in many cells**.

pRb functions by binding in the **G0/G1 phase** to proteins, some of which are **transcription factors** (e.g., **E2F**), which are active in the **S-phase** of the cell cycle. As a result, **the cell cycle is delayed**. In complex with **pRb**, these transcription factors remain **inactive**.

During the **G0** and **G1 phases**, **pRb** is **weakly phosphorylated**, while in the **late G1** and **early S phases**, its phosphorylation **increases**. **Phosphorylation of pRb** leads to **the release of transcription factors** (such as **E2F**), thereby **activating** them. These transcription factors then stimulate the expression of proteins that drive the cell cycle from **G1** to **S phase**.

The **hypophosphorylated form of pRb** can bind to **viral proteins**, forming a complex in which **pRb becomes inactive**. This reduces its **regulatory function** during **cell division**, helping explain **why certain viruses can transform cells**.

The **main types of cancer** where **pRb inactivation** plays a crucial role include *lung cancer, prostate adenocarcinoma, retinal tumors, bone tumors, and connective tissue tumors*.

Regulation of E2F by pRB

When **pRB** is **phosphorylated** by the **cyclin D/CDK4/6 complexes**, it results in the **release of E2F**, allowing **E2F** to become **active** and stimulate the **transcription** of specific genes that enable the cell to transition from the **G1** to the **S phase**.

E2F can activate the expression of **S-CDK (cyclin-dependent kinase) protein complexes**, such as **cyclin E/CDK2**, which further **phosphorylate pRB**, thereby maintaining the cell in a state favorable for cell cycle progression.

p53 – the "guardian of the genome"

p53 is a tumor suppressor gene that acts as the **"guardian of the genome"** (see Fig. 12-4). The **p53 protein** functions as a **transcriptional regulator** of genes primarily involved in cell division. **p53 binds to viral particles**, forming **inactive oligomeric complexes**. **p53** has at least three main effects:

1. **Transcriptional activation:** p53 regulates key genes involved in cell division.
2. **G1 checkpoint regulation for DNA damage:**
 - If DNA damage occurs, such as from UV radiation, p53 activity increases, leading to inhibition of cell division and allowing time for DNA repair.
 - If the cell cycle proceeds unchecked, DNA damage and mutations in the genome will be replicated.
 - If p53 is inactive, as seen in many tumors, it cannot perform this function, leading to genetic instability due to the accumulation of DNA mutations.
 - This is why p53 is known as the **"guardian of the genome"**.
3. **Initiation of apoptosis:**
 - In mature tissues, homeostasis is maintained by balancing cell growth and cell division.
 - p53 activation leads to the synthesis of p21, which regulates the retinoblastoma protein (Rb) (see Fig. 13-2).

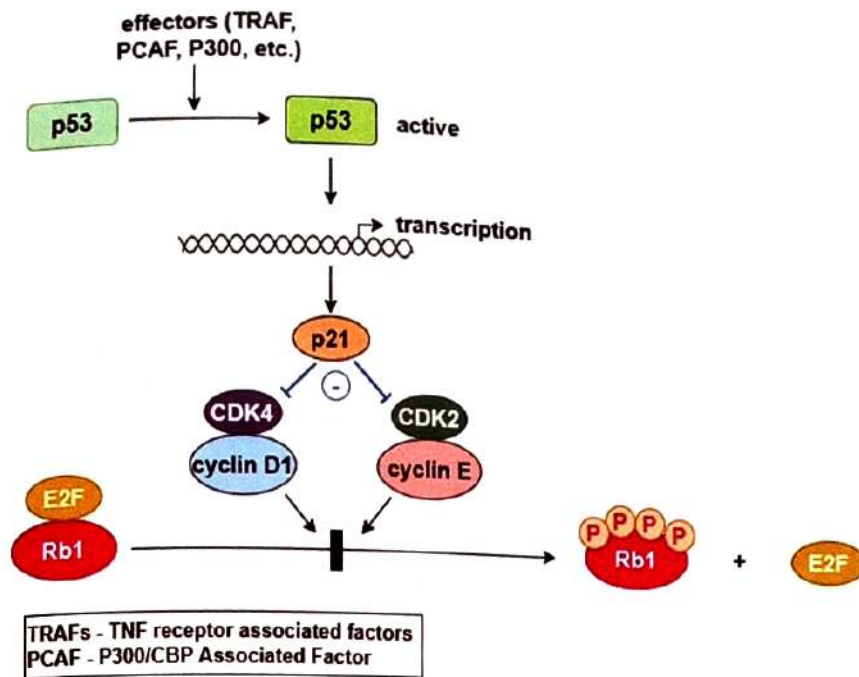


Fig. 13-2 p53 signaling pathway

p53 signaling pathway

In the downstream signaling pathway, p53 **arrests cell cycle progression in G1** by inducing **p21** transcription. p21 **prevents pRB phosphorylation** by inhibiting CDK4 and CDK2 kinases, effectively halting cell cycle progression.

Biochemical changes common in rapidly growing tumor cells

1. Increased activity of *ribonucleotide reductase*
2. Enhanced *DNA and RNA* synthesis
3. *Reduced* pyrimidine catabolism
4. Increased rate of *aerobic and anaerobic* glycolysis
5. Changes in isoenzyme profile, often towards a *fetal-like characteristic*
6. Synthesis of *embryonic proteins* (e.g., carcinoembryonic antigen)
7. Loss of certain biochemical functions (e.g., reduced synthesis of specialized proteins)
8. Inadequate synthesis of specific *growth factors and hormones*

Metastasis – the most dangerous feature of tumor cells

Metastasis refers to the **spread of tumor cells** from the **primary site** to other tissues, where they grow as **secondary tumors**.

The **loss of intercellular interactions** is facilitated by **proteases** (e.g., **collagenase**) and **glycosphingolipids** on the **cell surface**. Changes in oligosaccharide chains of glycoproteins (or alterations in the activity of specific **glycosyltransferases**) may be critical for **metastatic potential**.

Alterations occur in **proteins involved in cell-cell and cell-tissue interactions**, such as **integrins**, **cadherins**, and other **adhesion molecules** (e.g., **neural cell adhesion molecule – N-CAM**). **Integrins** can direct tumor cells towards specific organs for metastasis. They play a role in almost **every stage of cancer progression**, from **primary tumor formation** to **extravasation** and **metastatic site establishment**.

P-Glycoprotein and multidrug resistance in tumor chemotherapy

A major issue in **cancer therapy** is the development of **drug resistance** to the applied agent (**acquired resistance**).

The mechanism of multidrug resistance involves **P-glycoprotein (P-GP)**. In resistant tumor cells, an increased level of P-GP has been observed. P-GP is also present in **normal tissues** (e.g., kidneys, intestines). **Elevated P-GP levels in vivo correlate with poor prognosis**.

P-GP can be **induced by various stimuli**, including **heat shock**. Gene amplification and/or point mutations contribute to **multidrug resistance**.

P-GP acts as an **ATP-dependent efflux pump**, which **actively expels multiple drugs** from the cell, thereby contributing to **chemoresistance**.

Cancer and glycolysis

Tumors exhibit an **increased glucose uptake** and **accelerated glycolysis**.

Since **tumor cells grow faster than the blood vessels** that supply them, they experience **hypoxia** due to insufficient **oxygen delivery**. As a result, **anaerobic glycolysis** becomes the primary ATP source.

Glycolysis becomes more **efficient in hypoxic tumors** due to the action of a **transcription factor – hypoxia-inducible factor-1 (HIF-1)**. HIF-1 consists of two subunits: **HIF-1 α** and **ARNT** (aryl hydrocarbon receptor nuclear translocator).

In the **absence of oxygen**, **HIF-1 upregulates glycolytic enzymes** and **glucose transporters** (GLUT1 and GLUT3). Glucose uptake correlates with tumor progression and poor prognosis.

These **adaptations allow tumor cells to survive until vascularization (angiogenesis)** occurs. HIF-1 also promotes the **growth of new blood vessels** by upregulating the **vascular endothelial growth factor (VEGF)**, facilitating **tumor vascularization** (Fig. 13-3).

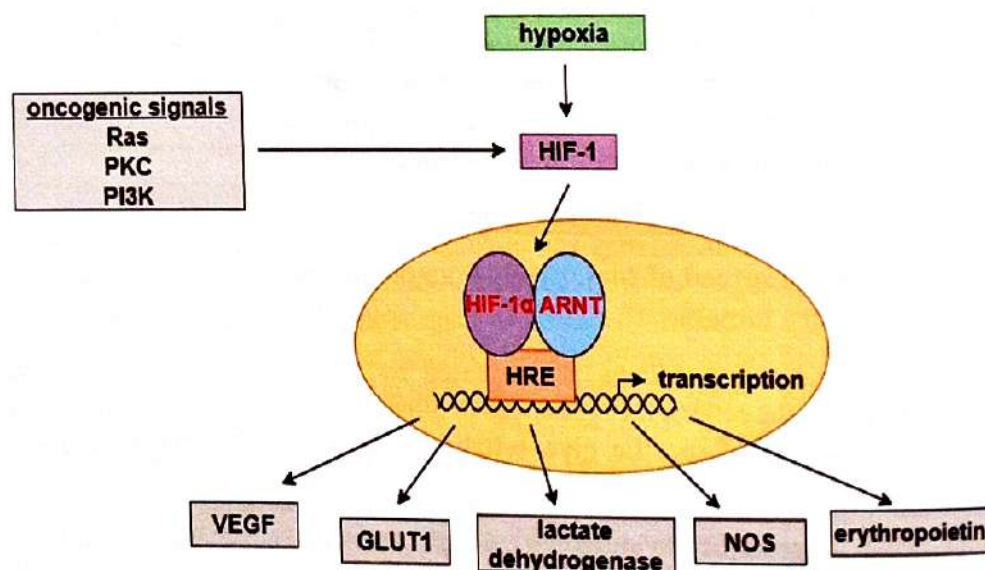


Fig. 13-3 Hypoxia – a key factor in tumor growth

Oncogenic proteins function as:
Growth factors (e.g., EGF)

Growth factors (e.g., EGF)

- Ras and Raf activate the ERK/MAP kinase pathway, leading to the induction of genes like Fos, which encodes oncogenic transcriptional regulators.**

Oncogenic proteins that promote cell survival include growth factors, growth factor receptors, PI3K, and Akt.

PI3K/Akt signaling regulates **Bcl-2** family members, **promoting cell survival** by inhibiting *cytochrome c* release from mitochondria.

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14. Pathobiochemical mechanisms of Diabetes Mellitus

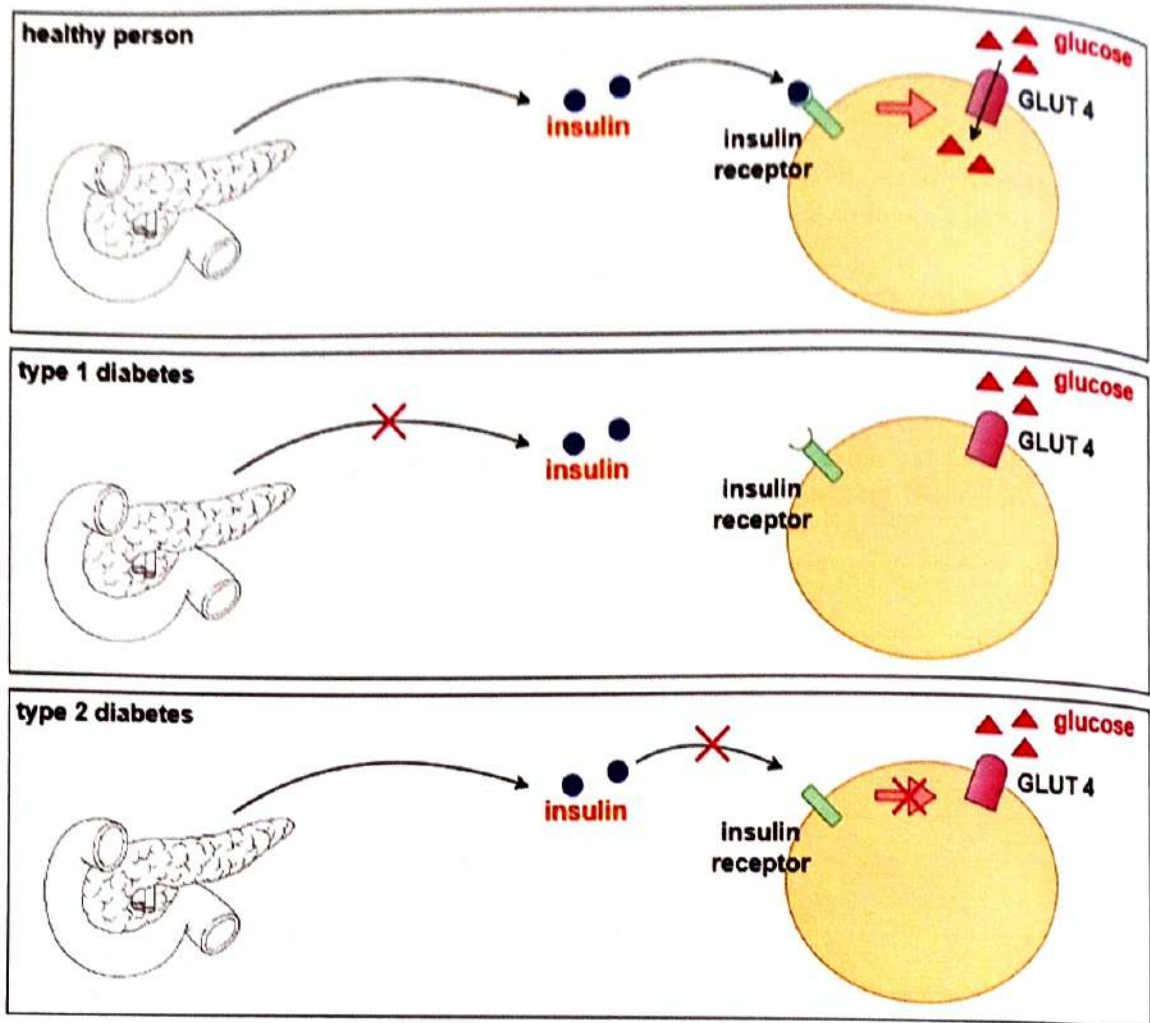


Fig. 14-1 Type I and type II diabetes mellitus

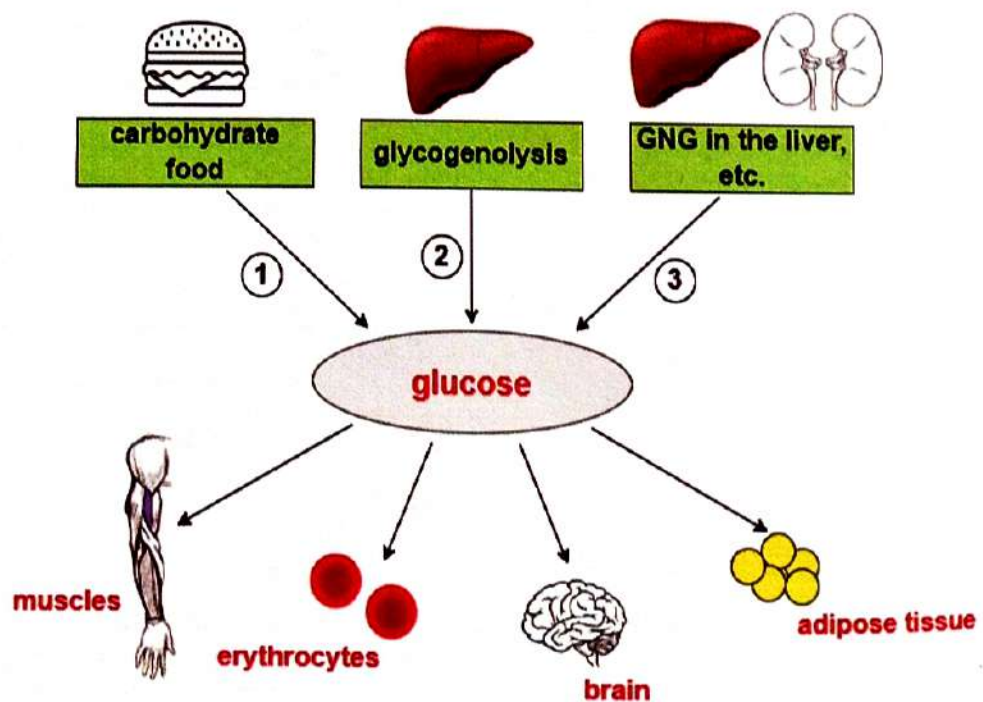


Fig. 14-2 Maintaining blood sugar levels

Blood glucose levels are maintained through three mechanisms: 1) **from carbohydrate intake**, 2) **from hepatic glycogenolysis** – between meals (short-term fasting periods, such as overnight fasting), and 3) **from gluconeogenesis (GNG)** – after 18-24 hours of fasting. Glucose is utilized by all cells, but primarily by **insulin-sensitive tissues** such as **muscle and adipose tissue**, as well as by **erythrocytes** (which lack mitochondria and cannot metabolize fatty acids (FA)) and the **brain** (which prefers glucose as an energy source since FA cannot cross the blood-brain barrier) (Fig. 14-2).

Definition: *Diabetes is a heterogeneous group of disorders characterized by chronic hyperglycemia and multiple metabolic, endocrine, and other disturbances due to an insufficient insulin effect.*

Depending on the cause of insulin deficiency, diabetes is classified into two main types – **Type 1 (T1DM)** and **Type 2 (T2DM)**, though other forms of diabetes also exist.

Type 1 diabetes mellitus (T1DM) – Insulin-dependent diabetes (Fig. 14-1)

It accounts for **10%** of cases and usually manifests **before the age of 30** due to **autoimmune** destruction of pancreatic β -cells in the islets of Langerhans, caused by **autoantibodies** against cytoplasmic components of islet cells, autoantibodies against insulin, autoantibodies against glutamate decarboxylase (GAD), autoantibodies against GM2-1 ganglioside, and autoantibodies against tyrosine phosphatase. **Genetic factors** also contribute to T1DM development, with genetic defects on chromosomes 6 and 11. Chromosome 6 contains the major histocompatibility complex (MHC), while chromosome 11 contains the insulin gene.

Type 2 diabetes mellitus (T2DM) – Non-insulin-dependent diabetes (Fig. 14-1)

It accounts for **90%** of cases and usually develops **after the age of 40** due to **insulin resistance** in **peripheral tissues**, which results from a lack or reduced number of insulin receptors, defective insulin receptors, autoantibodies against insulin receptors, or post-receptor defects that impair intracellular hormone signaling. **Insulin deficiency in T2DM is partial.**

Secondary or other specific types of diabetes mellitus include:

1. **Maturity-onset diabetes of the young (MODY)** – a **rare** form of diabetes (1% of all cases) that is often **misdiagnosed** as T1DM or T2DM. MODY affects **young** individuals (before age 25) but can develop at any age. It follows a dominant inheritance pattern and is characterized by **impaired β -cell function** and, in some cases, **insulin resistance**. MODY symptoms develop **gradually**. There are 11 known MODY types, each caused by mutations in different genes, with six being the most studied:
 - **MODY1:** Mutations in **hepatic nuclear factor-4 α** (HNF-4 α), essential for pancreatic growth and function. HNF-4 α regulates genes such as **insulin**, **glucose-6-phosphatase**, **GLUT2**, **liver pyruvate kinase** (L-PK), and **thermogenin** (UCP).
 - **MODY2:** Mutations in pancreatic **glucokinase**. The **mildest** form of MODY.
 - **MODY3:** The **most common type (70% of MODY cases)**, caused by mutations in HNF-1 α , which regulates HNF-4 α , GLUT2, and L-PK expression.
 - **MODY4:** Mutations in **IPF-1 (PDX1)**, a **transcription factor** crucial for **pancreatic development**.
 - **MODY5:** Mutations in HNF-1 β , which regulates **pancreatic growth and differentiation**. Associated with **pancreatic hypoplasia**, **kidney defects**, and **genital malformations**.

- **MODY6:** Mutations in **NeuroD1**, a gene involved in **neuronal fate determination** and **insulin transcription**.
- 2. **As a result of pancreatic diseases:** pancreatectomy, cystic fibrosis, pancreatitis.
- 3. **As a result of endocrine disorders:** Some tumors produce counter-regulatory hormones that antagonize insulin or inhibit insulin secretion, such as glucagon (glucagonomas), adrenaline (pheochromocytomas), cortisol (Cushing's syndrome), and growth hormone (acromegaly).
- 4. **Drug-induced diabetes:** glucocorticoids, thiazide diuretics, thyroid hormones.
- 5. **Latent autoimmune diabetes in adults (LADA):** A form of T1DM diagnosed in older adults (over 30 years old). Like T1DM, LADA results from autoantibody-mediated β -cell destruction.
- 6. **Gestational diabetes:** diabetes during pregnancy, which usually resolves after childbirth.

Insulin-Dependent Diabetes Mellitus Type I (T1DM)

Mechanisms of metabolic disorders in T1DM (general overview)

1. **The pancreas does not secrete insulin, only glucagon.**
2. In untreated diabetes, there is **hyperglycemia**, **hyperlipoproteinemia** (**chylomicrons and VLDL**), and episodes of **severe ketoacidosis**.
3. Hyperglycemia results from the **inability of insulin-dependent tissues to uptake glucose from the blood**, as well as from **increased gluconeogenesis** using amino acids derived from muscle proteins.
4. A **low insulin/glucagon ratio** leads to increased lipolysis in adipose tissue. This elevates fatty acid levels in the **blood and liver**. If **ketone bodies** are not utilized as quickly as they are produced, a state of **ketoacidosis** develops.

Symptoms of T1DM Due to Insulin deficiency

1. **Hyperglycemia.**
2. **Glycosuria** – when blood glucose concentration exceeds the maximum renal tubular reabsorption threshold of **9-10 mmol/L** (the so-called **renal threshold**), glucose passes into the urine.
3. The body becomes **dehydrated** due to **increased kidney activity** aimed at eliminating excess glucose through urine (**polyuria**). This leads to insatiable thirst (**polydipsia**).
4. Glycosuria causes a **significant loss of calories**.
5. The loss of muscle and fat tissue results in **weight loss**, despite **increased appetite** and higher food intake (**hyperphagia**).

Metabolic changes in T1DM

1. **Hyperglycemia**, due to **poor peripheral glucose uptake** and **excessive gluconeogenesis**.
2. **Ketoacidosis**, due to increased lipolysis and ketone body formation.
3. **Elevated fatty acid levels** lead to high VLDL content and hypertriglyceridemia. **Lipoprotein lipase** has **reduced** activity.

The effects of glucagon are not counteracted by insulin.

Mechanisms of Metabolic Disorders in T1DM

Cause of Hyperlipidemia:

1. Counter-regulatory hormones such as **glucagon** and **adrenaline** activate *hormone-sensitive adipocyte lipase*, leading to the **breakdown of TAG** in adipose tissue into **fatty acids**, which enter the blood.
2. Not all fatty acids that reach the **liver** can be processed through **β -oxidation** and **ketogenesis**. The remaining amount is used for the **synthesis of VLDL**. **Hypertriglyceridemia** occurs because **VLDL** are synthesized and released by the liver faster than they can be broken down by **lipoprotein lipase**, whose synthesis is **insulin-dependent**.

A deficiency of *lipoprotein lipase*, which is also necessary for **chylomicron catabolism**, leads to **hyperchylomicronemia**.

Cause of Ketoacidosis:

Ketoacidosis is a form of **metabolic acidosis** resulting from **high concentrations of ketone bodies**, are produced primarily from fatty acid oxidation. This occurs in **T1DM** when the liver breaks down **lipids and proteins** for **gluconeogenesis (GNG)** and synthesizes **ketone bodies** for energy. The **blood pH drops significantly**. In **T1DM patients**, **ketoacidosis** is typically accompanied by **dehydration, hyperglycemia, and insulin deficiency**.

Causes of increased ketone body formation in the liver

1. **Factors** that cause **increased lipolysis** and **mobilization of fatty acids** from adipose tissue (glucagon, adrenaline, pituitary hormones, etc.) lead to an **increased influx of fatty acids into the liver**.
2. These high fatty acid concentrations **exceed the liver's capacity** to esterify them into **TAG** or **phospholipids**, which primarily depends on the **supply of glycerol-3-phosphate** and the **presence of insulin**.
3. **Acetyl-CoA cannot be used** for the synthesis of fatty acids or cholesterol because **NADPH from the PPP is lacking and insulin is absent**.
4. The inhibitory effect of **malonyl-CoA** on **β -oxidation** is **removed**, as malonyl-CoA, a product of fatty acid synthesis, is an **inhibitor of the carnitine shuttle**. Malonyl-CoA is not formed because fatty acid synthesis is an insulin-dependent pathway that requires large amounts of **NADPH from the PPP**, which is non-functional in insulin and glucose deficiency.
5. **β -oxidation predominates**, leading to the **accumulation of acetyl-CoA**, which provides energy for **gluconeogenesis**.
6. **Acetyl-CoA cannot enter the TCA cycle**, which has a **limited capacity** in diabetes due to **oxaloacetate** being **diverted for gluconeogenesis, ammonia detoxification in the urea cycle, and the reversal of the oxaloacetate-malate reaction**.
7. The **only** remaining pathway for acetyl-CoA is its **conversion into ketone bodies**.

Although the symptoms of **insulin-dependent diabetes** appear suddenly, it develops **gradually and silently** until the **immune system destroys about 80% of the β -cells** in the pancreas. The remaining **20% are destroyed more rapidly**.

In **uncontrolled diabetes**, the **mortality rate** can reach up to **10%** due to **diabetic ketoacidosis and diabetic coma**.

Genetic factors in T1DM development

T1DM is a **multifactorial autoimmune disease**, with **genetic predisposition** determined by **genetic factors** and **environmental factors**. Autoantibodies are produced against **β -cell proteins** in the pancreatic islets of Langerhans, leading to **β -cell destruction** and **insufficient insulin production** or **total insulin deficiency**.

The **main cause** of autoantibody formation is **mutations in chromosome 6**, where the major histocompatibility complex (MHC) is located. **Mutations in the insulin gene on chromosome 11** are also significant. Other **genetic regions** in different **chromosomes** have been identified, contributing to diabetes susceptibility.

However, **genetic mutations alone** are considered **necessary but not sufficient** for diabetes onset. **Environmental factors**, many of which remain **unidentified**, also play a **crucial role**.

Environmental risk factors

Viral infection – can *trigger* type 1 diabetes through direct lysis of β -cells, by inducing an autoimmune response, or by disrupting the immune system balance. In most children carrying risk *MHC antigens DR and DQ*, disease onset is associated with viral infections.

Coxsackie viruses play a significant role. The 2C (P2C) protein from Coxsackie viruses shows a conserved homology region with **GAD** (glutamate decarboxylase) in the pancreas. However, GAD also shares **homologous sequences** with proinsulin, heat shock proteins, and carboxypeptidase H. Autoimmunity develops through **molecular mimicry**, where T-cells recognize similar sequences in proteins of different origins. Antibody production against foreign proteins that **resemble self-proteins** leads to an autoimmune response, where T-cells attack pancreatic β -cells.

Other possible factors include *toxic damage, poisoning, emotional stress, and dietary antigens (e.g., cow's milk proteins, gluten), as well as processed meats containing nitrosamines*. A **high glycemic index diet** increases insulin demand, forcing β -cells to produce more insulin, accelerating their destruction.

Type 2 Diabetes Mellitus (T2DM)

Mechanisms of metabolic disorders in T2DM (summary overview)

Unlike Type I diabetes, here the **insulin deficiency is partial**, meaning that **insulin is present**.

1. Hyperglycemia is due to the **reduced glucose uptake by peripheral tissues**, especially **muscles**.
2. **Ketoacidosis** usually **does not develop**, as there is **no uncontrolled lipolysis** in **adipose tissue**, because **insulin is present**.
3. **Hypertriglyceridemia** is present, but it is due to an **increase in VLDL**, without **hyperchylomicronemia**. The reason for the **elevated VLDL** is the **higher rate of synthesis in the liver**.

Insulin is secreted, but due to **insulin resistance**, **peripheral tissues** are unable to respond to its signal.

Insulin resistance can occur at any level of the insulin signaling pathway (**post-receptor defect**). The genes for the insulin receptor are located on chromosome 19.

Role of adipose tissue in insulin resistance development

Obesity often precedes T2DM and contributes to insulin resistance. Adipose tissue functions as an endocrine organ.

In obese individuals, there is **high TNF- α** expression in **adipose tissue**, which correlates with **insulin resistance**. TNF- α impairs insulin signaling by **phosphorylating IRS-1** and reducing **IRS-1 and GLUT4 expression**.

A **leptin deficiency**, another **adipose-derived hormone**, is also linked to **insulin resistance**. **Leptin restoration** improves glycemic control and reduces circulating lipid levels. **Resistin**, another adipose hormone, is elevated in diabetes and obesity.

Hypoglycemia

The **brain** critically depends on glucose (GLUT 1, 3, and 4).

A **glucose drop to 3 mmol/L** causes *adrenergic symptoms: headache, anxiety, palpitations, tremors, hunger, sweating, dizziness, and weakness*. A further drop to **2.6 mmol/L** leads to *cognitive dysfunction (speech and thinking difficulties, blurred vision)*. Levels around **1.7 mmol/L** cause *mild confusion and delirium*, while **1.1 mmol/L** leads to *stupor, seizures*, and below **0.6 mmol/L** results in *coma and death*.

Complications in Type 2 diabetes

Unlike patients with type I diabetes, who develop diabetic ketoacidosis, older patients with type II diabetes are more likely to develop **hyperglycemic hyperosmolar coma**. These patients **are unable to take insulin or glucose-lowering medications**, which worsens hyperglycemia and leads to water loss. The **circulating blood volume decreases**, and over a few days, **glucose levels can rise**, resulting in **dehydration and coma**.

Ketoacidosis **does not develop** because free fatty acids are not significantly increased, and **there is still some insulin in the blood**, which **inhibits lipolysis and ketogenesis**, but not **gluconeogenesis**.

Mortality in this condition is **higher** compared to **diabetic ketoacidosis**. Therapy aims to restore **fluid and electrolyte balance** and to correct **hyperglycemia**.

Complications possible in both types of diabetes

Hypoglycemia occurs due to an **imbalance between glucose production and utilization**.

Increased glucose utilization can occur in:

1. **Elevated insulin levels** due to pancreatic tumor (insulinoma), insulin injections, sulfonylurea intake, or lack of counterregulatory hormones.
2. **Increased IGF-II production** by a tumor.
3. A **very large tumor** that consumes glucose for energy.

Decreased glucose production can occur in:

1. **Liver and kidney damage** (the main organs for **gluconeogenesis**).
2. **Alcoholism** (ethanol reduces a large portion of **cellular NAD** and interferes with **gluconeogenesis**).
3. **Glycogen storage diseases** in the liver or muscles, preventing the breakdown of glycogen into glucose.

Pathological consequences of hyperglycemia

1. Increased susceptibility to **infections** due to immune system dysfunction.
2. Increased **oxidative stress**.
3. Endothelial **dysfunction**.
4. Increased **coagulation**.
5. Increased **inflammation**.

Pathobiochemical mechanisms of diabetes complications

1. Activation of the **sorbitol (polyol) pathway**.
2. Increased non-enzymatic **protein glycation**.
3. Activation of the **DAG/PKC cascade**.
4. **Oxidative stress**.
5. Stimulation of the **hexosamine metabolism**.

Significance of the polyol pathway and protein glycation in micro- and macrovascular damage (Fig. 14-3)

The lens, peripheral nerves, renal papillae, glomeruli, and retinal capillaries contain two enzymes involved in the **polyol pathway** – *aldose reductase* and *sorbitol dehydrogenase* (see Fig. 3-13 from Medical Biochemistry Part I).

The first enzyme requires **NADPH**, while the second requires **NAD⁺**. In diabetes, sorbitol levels **increase in these tissues**.

Sorbitol causes **tissue swelling** – it does not diffuse easily through the **cell membrane**, accumulating and causing **osmotic damage**.

Another **metabolic impairment** in nerves is the reduction of **myo-inositol**, a component of **membrane phosphatidylinositol** and the **second messenger inositol triphosphate**.

The **polyol pathway** leads to **microvascular damage** (retinopathy and nephropathy) and **neuropathies**, as well as **macrovascular damage**, affecting the **heart, brain, peripheral vessels, and nerves**.

In **late stages of untreated diabetes**, **blindness** and **renal failure** develop. The latter, along with **myocardial infarction**, are the leading causes of **mortality in diabetes**.



Fig. 14-3 Polyol pathway

Etiology of microvascular disorders in diabetes

Increased **activation of the polyol pathway** raises the **NADH/NAD⁺ ratio**, increasing **protein kinase C (PKC) activity**, decreasing **glyceraldehyde-3-phosphate dehydrogenase activity**, and **reducing pyruvate formation**.

Excess **glyceraldehyde** induces **non-enzymatic protein glycation**, altering the **structure and function of extracellular matrix proteins**, such as those in the **vascular basement membrane**. It also interacts with **intracellular receptors**, triggering **prothrombotic changes** in the **endothelial surface** through the release of **cytokines and growth factors**.

Non-enzymatic protein glycation

1. Early Glycation – Glucose reacts non-enzymatically with a large number of proteins, forming intermediate products (Amadori or fructosamine). This is particularly characteristic of hemoglobin, though almost any protein can be affected. In clinical practice, the measurement of glycated hemoglobin (HbA1c) has revolutionized diabetes monitoring and metabolic control assessment. HbA1c is a marker of long-term metabolic control, reflecting glucose levels over the past three months. This period corresponds to the lifespan of erythrocytes, which is approximately 100–120 days. Hemoglobin undergoes non-enzymatic glycation in the presence of elevated blood glucose levels. HbA1c levels $\geq 6.5\%$ indicate diabetes (Table 14-1).

Table 14-1 Correlation between blood glucose level and HbA1c content determined in erythrocytes of healthy and diabetic patients

HbA1c	Glucose level (mmol/L) over the previous 2-3 months
5 %	5.4
6 %	7.0
7 %	8.6
8 %	10.2
9 %	11.8
10 %	13.4
11 %	14.9
12 %	16.5

Fructosamine (chemically, these are proteins bound to fructosamine) is a general term for all glycated plasma proteins in the first stage of glycation. The measurement of glycated plasma proteins, such as albumin, is commonly referred to as "fructosamine testing" and is used to monitor glycemic control over a three-week period.

2. Second Phase of the Glycation Pathway – A series of rearrangement and oxidation reactions occurs, leading to the formation of multiple highly reactive compounds, collectively known as "advanced glycation end products" (AGEs).

A similar reaction occurs in food, where proteins and sugars interact. This process is known as the Maillard reaction and plays a role in the formation of the brown pigment in beer and cola.

Reactive dicarbonyl precursors, derived from dicarbonyl intermediates, react with amine groups of proteins, leading to the formation of diverse AGEs.

AGE products accumulate in vivo on vascular collagen and basement membranes, depending on age and glycemia levels. They are capable of forming cross-links with proteins and exert various biological activities.

AGE and Atherosclerosis

Potential mechanisms by which LDL glycation increases its atherogenicity

Unlike fibroblasts, macrophages recognize glycated LDL to a greater extent than native LDL. However, the uptake of glycated LDL by these cells is not mediated by the LDL receptor, but rather by scavenger receptors—high-capacity, low-affinity receptors. LDL glycation correlates with glucose levels, and AGEs-ApoB levels are up to four times higher in diabetics. Glycation increases the susceptibility of LDL to oxidative modifications, which is considered a key step in atherogenesis. Macrophages transform into foam cells. The uptake of glycated LDL through the scavenger receptor is believed to trigger intracellular cholesterol ester accumulation, leading to the development of atherosclerosis. This may also increase the sensitivity of the diabetic endothelium to the release of growth factors and cytokines.

Receptor-mediated effects of AGE - RAGE (Receptor for Advanced Glycation End Products)

AGEs are recognized by receptors called **RAGE**, which can increase endothelial permeability, stimulate the proliferation of vascular smooth muscle cells, induce the formation of reactive oxygen species (ROS), and initiate the pro-inflammatory NF- κ B pathway in vascular endothelial cells, as well as promote procoagulant effects. In macrophages, they can induce the expression of pro-inflammatory cytokines (e.g., TNF- α , IL-1). The interaction of AGEs with RAGE-expressing endothelial cells mediates the initial events of atherosclerosis and plays a significant role in the pathogenesis of **diabetic vascular disorders**.

Protein Kinase C and diabetes

Hyperglycemia activates **PKC**, increasing the production of diacylglycerol (DAG) from glycolytic intermediates such as dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. DAG is the primary endogenous cellular activator of PKC.

Increased DAG levels and subsequent PKC activation in blood vessels can be maintained **chronically**. In vascular smooth muscle cells, PKC activation leads to increased expression of **PDGF- β receptors** (platelet-derived growth factor- β).

PKC activation also increases the expression of **TGF- β** (transforming growth factor- β), one of the most important growth factors regulating **extracellular matrix formation** by enhancing the gene expression of proteoglycans and collagen while reducing the synthesis of proteases that degrade matrix proteins. Increased TGF- β expression may result in the **thickening of the capillary basement membrane**, an early structural abnormality in almost all diabetic tissues.

Oxidative stress

Oxidative stress, a major consequence of hyperglycemia, is considered a potential mechanism for accelerating atherosclerosis.

A **primary mechanism** involves the intracellular production of reactive oxygen species (ROS), generated by the proton electrochemical gradient formed by the **respiratory chain**, leading to increased superoxide anion production.

Glucose tolerance test for healthy and diabetic individuals

Criteria for clinical confirmation of diabetes include (Table 14-2):

- Fasting plasma glucose levels above 7 mmol/L
- Plasma glucose levels above 11.1 mmol/L at two time points during an oral glucose tolerance test (OGTT), one of which must be 2 hours after glucose intake.

Table 14-2 Criteria for impaired glucose tolerance and diabetes according to WHO

	two hours after glucose load	fasting blood sugar
normal	< 7.8 mmol/L	< 6.1 mmol/L
impaired fasting glucose	< 7.8 mmol/L	> 6.1 < 6.9 mmol/L
impaired glucose tolerance	> 7.8 mmol/L	< 7 mmol/L
diabetes	> 11.1 mmol/L	> 7 mmol/L



15. Biochemistry of blood

Biomedical Significance of Blood

1. The **functional role of blood** is to **maintain homeostasis**.
2. Changes in the **serum protein content** are observed in many diseases and can be monitored using **electrophoresis**.
3. Alterations in the **activity of specific enzymes** found in plasma have **diagnostic significance** in various pathological conditions.
4. **Hemorrhages** and **thrombotic conditions** require serious medical intervention and, in many cases, can have a **fatal outcome**.
5. The primary functions of blood cells are **oxygen transport** to tissues (**erythrocytes**) and **cell-mediated immune response** (**white blood cells**).
6. **Blood plasma** consists of water, electrolytes, metabolites, nutrients, proteins, lipoproteins, growth factors, and hormones. The electrolyte and water composition of plasma is similar to that of all extracellular fluids.

Blood Cells

Erythrocytes (RBCs)

- Erythrocytes **transport oxygen** in the blood. Their shape is ideal for this function. Viewed from above, they appear **round**, but a side view reveals their **biconcave disc shape**. This shape increases the surface area-to-volume ratio, enhancing the diffusion efficiency of oxygen and carbon dioxide into and out of the cell. Erythrocytes have a **flexible** plasma membrane, allowing them to pass easily through capillaries.
- To create more space for hemoglobin, which carries more oxygen, erythrocytes **lose their nucleus and other organelles** during differentiation into mature cells. Due to the lack of cellular repair mechanisms, they have a limited lifespan of approximately **120 days**.
- Erythrocytes are the most abundant cell type in the body and **die at a rapid rate (2–3 million per second)**. Their production must **equal** their destruction to maintain the population. They are produced through **erythropoiesis**.
- The removal of old and dying erythrocytes occurs in the **reticuloendothelial system** (**macrophages**).

Erythropoiesis

Under **low oxygen** levels, the transcription factor **HIF-1 (hypoxia-inducible factor-1)** is activated, leading to the expression and production of the hormone **erythropoietin (Epo)** in the kidneys. Erythropoietin then binds to its receptor (EpoR).

Erythropoietin has pleiotropic effects:

Main function: Stimulates erythrocyte production in the bone marrow. As the number of erythrocytes increases, the oxygen-carrying capacity of the blood also increases.
Other functions of Erythropoietin (Epo): *effects on endothelial cells, neurons, and glial cells.*

Key signaling pathways of Erythropoietin:

- Activation of **EpoR** leads to phosphorylation of **Janus tyrosine kinase 2 (Jak2)**, which triggers other signaling molecules, including **p38 MAPK** (p38 mitogen-activated protein kinase), **ERK1/2** (extracellular signal-regulated kinase 1/2), **STAT5**, and **PI3K**.
- Additionally, **Akt** and **nuclear factor- κ B (NF- κ B)** are also activated.

Hematocrit (Fig. 15-1)

Whole blood consists of **plasma (fluid), cells, and platelets**. If blood is placed in a capillary tube and **centrifuged**, the cells and plasma separate.

Erythrocytes (RBCs), being the heaviest, settle at the **bottom**, while plasma remains at the top. **Leukocytes and platelets** form a thin layer between RBCs and plasma.

Hematocrit is defined as the **percentage** of erythrocytes in whole blood. It is calculated by dividing the height of the erythrocyte column by the total blood height in the tube and multiplying by 100.

Hematocrit levels vary depending on **gender and environmental conditions**, but there is a range of values considered normal:

- Men: 40–54%
- Women: 37–47
- Athletes: > 50%

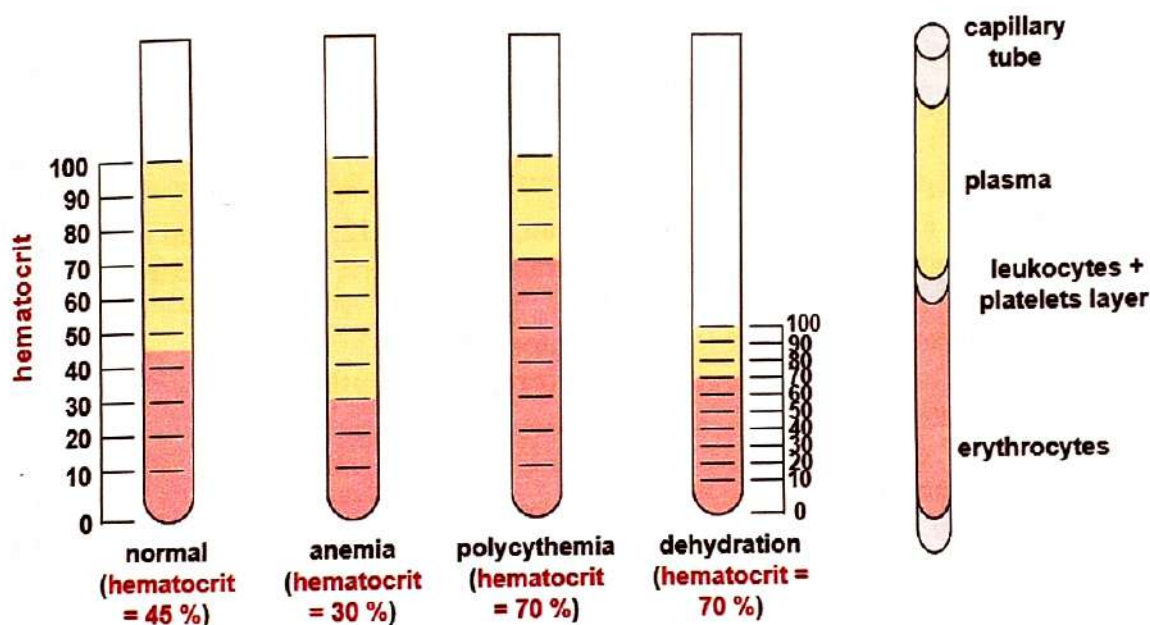


Fig. 15-1 Hematocrit

Factors that increase hematocrit

1. *Physical exercise* – During **aerobic exercise**, oxygen levels in the blood decrease due to **increased oxygen consumption** by active skeletal muscles. This stimulates **erythropoiesis**, increasing **hematocrit and the oxygen-carrying capacity** of the blood. Regular aerobic training raises hematocrit levels.
2. *Living at high altitudes* – The number of erythrocytes increases to **compensate for lower oxygen content** in the air.
3. *Injection of recombinant erythropoietin* – Some athletes use erythropoietin **illegally** to increase hematocrit and enhance **endurance**. It is also used for **treating chronic anemia associated with chronic kidney disease**.
4. *Dehydration* – The most common cause of high hematocrit. It leads to a **reduced volume of blood plasma**, resulting in a **relative increase** in erythrocyte concentration.
5. *Polycythemia vera* – A rare **bone marrow disorder** in which **more erythrocytes than normal** are produced.

6. *Lung diseases* – An attempt to **compensate for impaired oxygen exchange** by producing more erythrocytes.
7. *Conditions leading to hypoxia (low oxygen levels in the blood)* – Examples include **premature infants with delayed lung development**.

Erythrocyte enzymopathies

- There are more than **14 known erythrocyte enzymopathies** (9 from the **glycolytic pathway** and 5 from the **pentose phosphate pathway (PPP)**), which result in **hemolytic**.
- The most common deficiencies involve:
 - *Glucose-6-phosphate dehydrogenase (G6PD) deficiency (favism)*;
 - *Pyruvate kinase deficiency*;
 - *Methemoglobin reductase deficiency* – prevents the reduction of Fe^{3+} to Fe^{2+} in hemoglobin, leading to **methemoglobinemia**, impaired oxygen transport, and tissue hypoxia.

Anemia

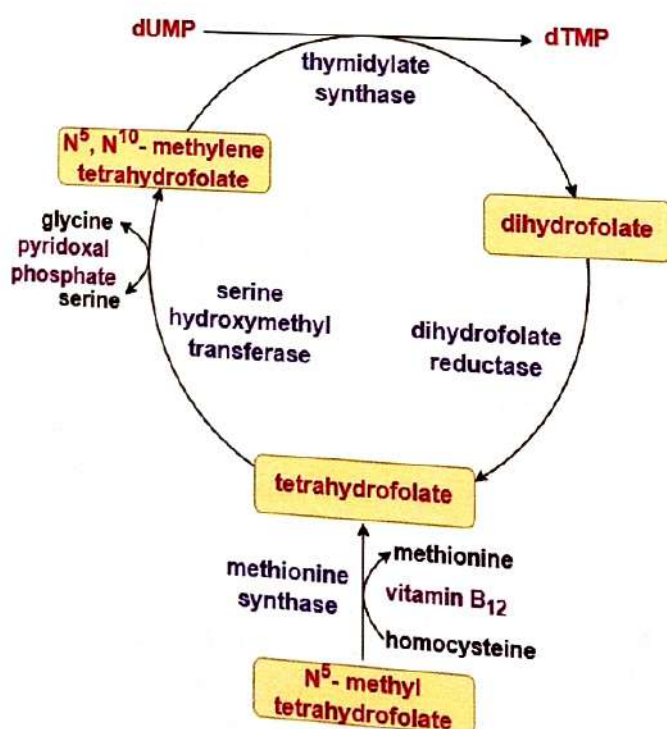
Anemia is a condition characterized by **reduced oxygen-carrying capacity** of the blood. It results from **low hemoglobin levels**, a **decreased number of erythrocytes (low hematocrit)**, or both.

Causes of Anemia:

1. **Deficiency or impaired absorption of iron, vitamin B12, or folic acid** in the diet.
2. **Hemorrhage** – caused by **injury, blood donation, ulcers, heavy menstruation**, etc.
3. **Hemolysis** – destruction of erythrocytes.
4. **Bone marrow failure** – due to **cancer or toxic drugs**.
5. **Kidney disease** – leading to **reduced erythropoietin synthesis**.

Symptoms of Anemia:

- Fatigue
- Increased heart rate
- Shortness of breath
- Low blood pressure
- Pale skin



Vitamin B12 and folic acid are required for **DNA synthesis (dTMP)** before cell division (Fig. 15-2).

- Without these vitamins, erythrocyte production **decreases**.
- **Hematocrit** levels drop, and erythrocytes become large and fragile (called **macrocytes**).
- Vitamin B12 deficiency leads to "**pernicious (megaloblastic) anemia**".

Fig. 15-2 Participation of folic acid and vitamin B12 in DNA synthesis

Metabolism of Erythrocytes

In the highly simplified structure of a mature erythrocyte, metabolic processes are minimized. The only energy source is **anaerobic glycolysis**, which utilizes 90% of glucose. As a byproduct of glycolysis, **2,3-biphosphoglycerate** is produced, which acts as a **regulator of oxygen affinity to hemoglobin**.

The remaining 10% of glucose is metabolized through the **pentose phosphate pathway (PPP)**, which provides **NADPH**. NADPH is necessary to maintain hemoglobin in its **reduced state (Fe^{2+} ferrous hemoglobin)** and to keep glutathione **reduced**. Oxidized hemoglobin (Fe^{3+}) – **methemoglobin** is functionally **inactive**, and normally, its concentration in the blood does not exceed 1–2%.

The **main metabolic pathways in erythrocytes** include:

- Embden-Meyerhof pathway (anaerobic glycolysis)
- Rapoport-Luebering shunt (also known as the *hexose monophosphate shunt* or PPP)
- Glutathione pathway
- Nucleotide metabolism (for free nucleotides)

Structural organization of the erythrocyte membrane (Fig. 15-3)

Spectrin forms **$\alpha_2\beta_2$ tetramers** and binds to short **actin** oligomers and **protein 4.1** in a complex that facilitates its interaction with **band 3 protein** in the membrane.

The link between spectrin and actin is initiated by **adducin**, **tropomodulin**, **tropomyosin**, and **glycophorin**.

A second connection to band 3 and the membrane is formed through the **ankyrin complex**, which is attached to spectrin.

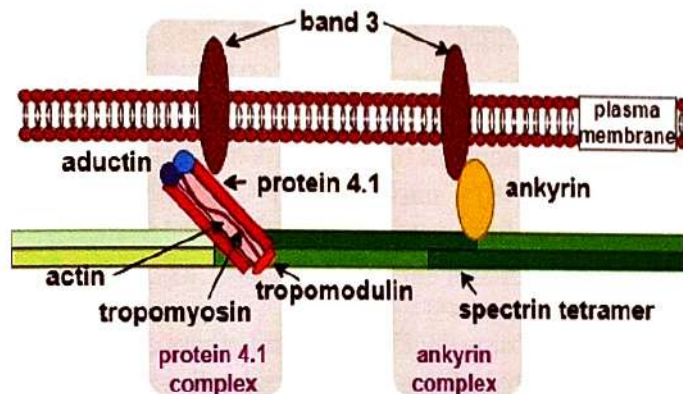


Fig. 15-3 Structure of the erythrocyte membrane

Leukocytes

Role and metabolism of leukocytes

Leukocytes (white blood cells) are involved in **cellular immunity**, which can be **specific or nonspecific**.

Leukocytes include:

- *Neutrophils*
- *Eosinophils*
- *Basophils*
- *Lymphocytes*
- *Monocytes*

Although leukocytes constitute only about **1% of blood**, they play a crucial role in defending the body against **infections/diseases** caused by various microorganisms. Additionally, they participate in **allergic** reactions, contribute to the **repair** of damaged tissues, and are involved in the **elimination of tumor** cells. However, in some cases, leukocytes can cause diseases, such as **autoimmune** disorders. During **phagocytosis**, the energy required for active motility of leukocytes comes from **ATP**. Their movement is achieved through the folding and unfolding of fibrillar proteins.

Active metabolic processes include:

- *Glycolysis*
- *Citric acid cycle (TCA cycle)*
- *Electron transport chain (respiratory chain)*
- *Pentose phosphate pathway (PPP)*
- *Lipid metabolism*
- *Protein biosynthesis*
- *Enzymatic reactions related to bactericidal activity*

Stages of Phagocytosis

I. Pathogen engulfment

- **Neutrophils and macrophages** engulf pathogens into a **phagosome**, which then fuses with lysosomes, forming a **phagolysosome**.

II. Microbial destruction

Several factors contribute to pathogen destruction within the phagolysosome:

- 1. Reactive oxygen species (ROS)** – generated by *NADPH oxidase* and further converted by *superoxide dismutase* (SOD) and *myeloperoxidase* (MPO) into bactericidal molecules such as superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hypochlorous acid (HOCl).
- 2. Reactive nitrogen species (RNS)** – nitric oxide (NO), produced by *nitric oxide synthase* (NOS), reacts with superoxide to form peroxynitrite ($ONOO^-$), a highly reactive oxidant that damages bacterial components.
- 3. Antimicrobial proteins and enzymes:**
 - *Proteases*: Elastase, cathepsins (B, D, G, L), and others degrade bacterial proteins.
 - *Glycosidases*: Lysozyme hydrolyzes the bacterial cell wall.
 - *Defensins*: Disrupt bacterial membranes.
- 4. Iron- and metal-binding proteins:**
 - *Lactoferrin* sequesters Fe^{3+} , limiting bacterial growth.
 - *Calprotectin* binds Zn^{2+} and Mn^{2+} , inhibiting bacterial metabolism.
- 5. Proton transport systems:**
 - *Vacuolar ATPase* (V-ATPase) actively pumps H^+ ions into the phagolysosome, lowering pH and activating lysosomal proteases.
 - *Chloride transporters* facilitate HOCl production via MPO, enhancing bacterial killing.

III. Release of regulatory molecules

Regulatory molecules that **modulate immune responses** are called **cytokines**. They are primarily released by **white blood cells**, especially from macrophages: **TNF- α** , **IL-1 β** , **IL-6**.

Cytokines mainly act in a **paracrine** manner, but they also have **systemic** effects. For example, some cytokines diffuse to the site of infection and contribute to **fever** (IL-1 β , IL-6, TNF- α).

IV. Pathogen recognition

Phagocytosis is triggered when particles covered with opsonins interact with specific receptors on the phagocyte surface. These receptors include:

1. **Fc receptors (FcR)** – bind to the Fc region of immunoglobulins (Ig).
 2. **Complement receptors** – recognize complement-coated particles.
 3. **Toll-like receptors (TLRs)** – recognize pathogen-associated molecular patterns (PAMPs) and initiate innate immune responses.
- **TLR4** binds to bacterial **lipopolysaccharides (LPS)**, leading to the activation of protective mechanisms in phagocytic cells.

When opsonized particles bind to these receptors, they are internalized into the **phagosome**. The interaction of FcR with immunoglobulin ligands initiates a series of leukocyte responses that play a crucial role in inflammation and immunity. These responses include:

- ✓ **Phagocytosis** – Engulfment and intracellular degradation of pathogens within the phagolysosome.
- ✓ **Antibody-dependent cellular cytotoxicity (ADCC)** – Fc receptor-mediated lysis of infected cells by NK cells, neutrophils, or eosinophils.
- ✓ **Release of pro-inflammatory mediators** – Secretion of histamine, prostaglandins, leukotrienes, and chemokines, which amplify the inflammatory response.
- ✓ **Cytokine production** – Activation of interleukins (**IL-1**, **IL-6**), tumor necrosis factor-alpha (**TNF- α**), and interferon-gamma (**IFN- γ**), which regulate inflammation and adaptive immunity.
- ✓ **Respiratory burst (oxidative burst)** – Activation of **NADPH oxidase**, leading to the production of reactive oxygen species (**ROS**) (e.g., superoxide, hydrogen peroxide, hypochlorous acid) to kill pathogens.
- ✓ **NETosis (Neutrophil Extracellular Traps, NETs)** – Neutrophils release **DNA**, **histones**, and **antimicrobial proteins**, forming extracellular traps that immobilize and neutralize bacteria.
- ✓ **Inflammasome activation** – The **NLRP3 inflammasome** activates **caspase-1**, triggering the secretion of **IL-1 β** and **IL-18**, which enhance the inflammatory response.

T-Lymphocytes

Metabolism of Lymphocytes

T-cells use **glucose** and **glutamine** (which is converted into α -ketoglutarate and enters the TCA cycle) as their primary energy sources, with minor utilization of **ketone bodies** and **fatty acids**.

Glucose serves as the main substrate for ATP production, which is necessary for the synthesis of nucleic acids, phospholipids, and other metabolites. Additionally, glucose is metabolized via the **pentose phosphate pathway (PPP)** to supply NADPH and ribose-5-phosphate.

Resting (non-activated) T-cells require extracellular signals, such as cytokines, hormones, and growth factors, to uptake sufficient glucose for sustaining their vital functions. Resting T-cells internalize and degrade GLUT1 and thus cannot maintain their viability.

Activated T-cells have drastically increased metabolic demands to support energy and biosynthetic needs required for growth, proliferation, and effector functions. **Aerobic glycolysis** becomes the **primary energy source**. Therefore, T-cell activation leads to an **increase in GLUT1 expression** and its localization on the **cell surface** (Table 15-1).

Table 15-1 Metabolic characterization of resting T cells and activated T lymphocytes

	non-activated T cells	activated T cells
metabolic characteristic	catabolism	anabolism
main source of ATP formation	oxidative phosphorylation	glycolysis
absorption of energy substrates	low	high rate of glucose and amino acid absorption
glycolysis rate	low	high
biosynthesis processes	to maintain homeostasis	increased synthesis of lipids, proteins and nucleic acids
catabolic processes	breakdown of glucose, amino acids, and lipids to form ATP	inhibition of β -oxidation; amino acid synthesis
growth profile	progressive atrophy without underlying extracellular signaling	exponential growth and proliferation

If **glucose uptake is limited**, glycolysis decreases to a level that no longer supports viability, leading to the activation of **pro-apoptotic members of the Bcl-2 family** (such as Bax and Bak), ultimately inducing apoptosis. On the other hand, **excessive glucose uptake** can trigger a hyperactive immune response (IL-2, IFN- γ , TNF- α , IL-6).

This is particularly relevant in **diets rich in high-glycemic index (GI) foods**, which cause rapid spikes in blood glucose levels, leading to increased glycolytic flux, activation of the mTOR pathway, and elevated production of pro-inflammatory cytokines (e.g., IL-6, TNF- α).

Chronic consumption of high-GI foods has been linked to persistent low-grade inflammation, oxidative stress, and an increased risk of autoimmune and metabolic disorders.

Regulation of T-Lymphocyte metabolism

A key regulator of protein synthesis and overall metabolic control in T-cells is the **mammalian Target of Rapamycin (mTOR) kinase**. mTOR modulates the rate of protein synthesis by sensing **nutrient availability (amino acids (Leucine), glucose, and lipids), growth factors, and energy status**.

The **serine/threonine kinase mTOR** is regulated by **upstream signaling pathways**, including the **PI3K/AKT pathway**, which activates mTOR in response to growth signals, and the **AMP-activated protein kinase (AMPK) pathway**, which **inhibits** mTOR under low-energy conditions. AMPK acts as a **metabolic checkpoint**, **suppressing** mTOR activity when ATP levels are low, thereby limiting excessive T-cell activation and biosynthesis.

mTOR is essential for T-cell differentiation:

- **mTORC1** activation promotes effector T-cell (Teff) differentiation, including Th1, Th2, and Th17 subsets.
- **mTORC2** is involved in the regulation of cytoskeletal organization and survival of activated T cells.
- Regulatory T cells (Tregs) require balanced mTOR signaling, as excessive activation impairs their immunosuppressive function.

Plasma proteins

Blood plasma contains a **complex mixture of proteins**, including:

- *Proteins*
- *Glycoproteins*
- *Lipoproteins*

It also contains **thousands of antibodies**, although, under normal conditions, **each antibody is present at very low concentrations**.

When plasma proteins are separated using **ammonium sulfate precipitation**, they are classified into **three groups**:

1. *Fibrinogen*
2. *Albumin*
3. *Globulins*

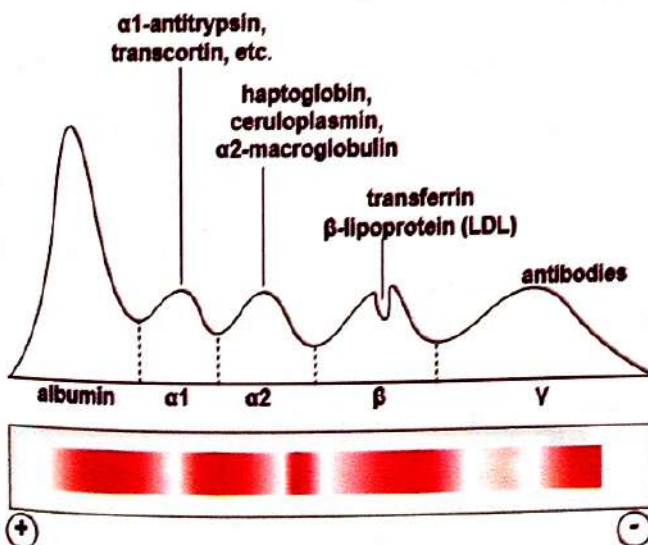
The most common method for analyzing plasma proteins is **electrophoresis**. In clinical laboratories, electrophoresis is performed on a cellulose acetate membrane. After electrophoresis and appropriate staining, **plasma proteins separate into five bands (fractions)**:

- **Serum albumin**
- **Serum globulins**, which migrate in the following order:
 - **α 1- and α 2-globulins** – *thyroxine-binding globulin, retinol-binding protein*
 - **β -globulins** – e.g., *transferrin, hemopexin*
 - **γ -globulins** – *antibodies*; γ -globulins increase during **infections or immunizations**

Serum Proteins

Serum is **blood plasma without fibrinogen** (which has been converted into **fibrin**) and **other coagulation factors**. When **venous blood is collected and allowed to clot**, the clear fluid remaining above the clot is called **serum**.

The **stained cellulose acetate plate** used in electrophoresis is called an **electrophoregram**.



The content of **protein fractions** can be determined using **scanning densitometry** attached to the electrophoresis device (densitogram). In many diseases, abnormalities in **one or more electrophoretic bands** can be detected (Fig. 15-4).

Fig. 15-4 Densitogram and electrophoregram

Characteristic features of plasma proteins

1. *Most plasma proteins are synthesized in the liver.* Exceptions include:

- γ -globulins, which are synthesized by **plasma cells**
- Some proteins synthesized by **endothelial cells** - endothelin-1, vWF, etc.

2. *Many plasma proteins are synthesized as precursors, known as **preproteins**, which contain **signal peptides**.* These precursors undergo **post-translational modifications** such as **proteolysis, glycosylation, phosphorylation**, etc.

3. *Almost all plasma proteins are **glycoproteins**, containing N-linked and/or O-linked oligosaccharide chains.* The notable exception is **albumin**, which **lacks carbohydrate residues**.

4. *Many plasma proteins exhibit **polymorphisms**, meaning they have at least two phenotypes that occur frequently.* The **ABO blood group antigens** are well-known examples, but other **plasma proteins** also exhibit polymorphisms, such as:

- **α 1-antitrypsin**
- **Transferrin**
- **Ceruloplasmin**
- **Immunoglobulins**

5. *Each plasma protein has a **characteristic half-life**.*

In certain diseases, **plasma protein half-life** can be significantly altered. For example, in **Crohn's disease** (an autoimmune inflammatory bowel disease), **large amounts of plasma proteins may be lost** through the **inflamed intestinal mucosa**. Patients with this condition develop **protein-losing gastroenteropathy**, and the **half-life of injected iodine-labeled albumin** can be **reduced to just one day**.

6. *The levels of certain plasma proteins increase during **acute inflammatory states**, secondary to **specific tissue injuries** and in **chronic inflammatory diseases** such as **cancer**.* These proteins are called **acute-phase proteins** (Fig. 15-5) and include:

- C-reactive protein (CRP)
- Serum amyloid A (SAA)
- α 1-antitrypsin, α 1-acid glycoprotein
- Haptoglobin, Hemopexin, Ceruloplasmin
- Complement components (e.g., C3, C4)
- Fibrinogen
- Ferritin

The increase in these proteins may range from **50% to 1000-fold**, as seen in **CRP**. It is believed that these proteins help the body **respond to inflammation**. For example:

- **CRP activates the classical complement pathway**
- **α 1-antitrypsin neutralizes specific proteases released during inflammatory conditions**

Macrophages and neutrophils **release various cytokines**, such as:

- **Interleukins IL-1, IL-6, IL-8**
- **Tumor Necrosis Factor- α (TNF- α)**

These cytokines enter the bloodstream in response to **injury and tissue damage** and stimulate **hepatocytes** to produce **acute-phase proteins**.

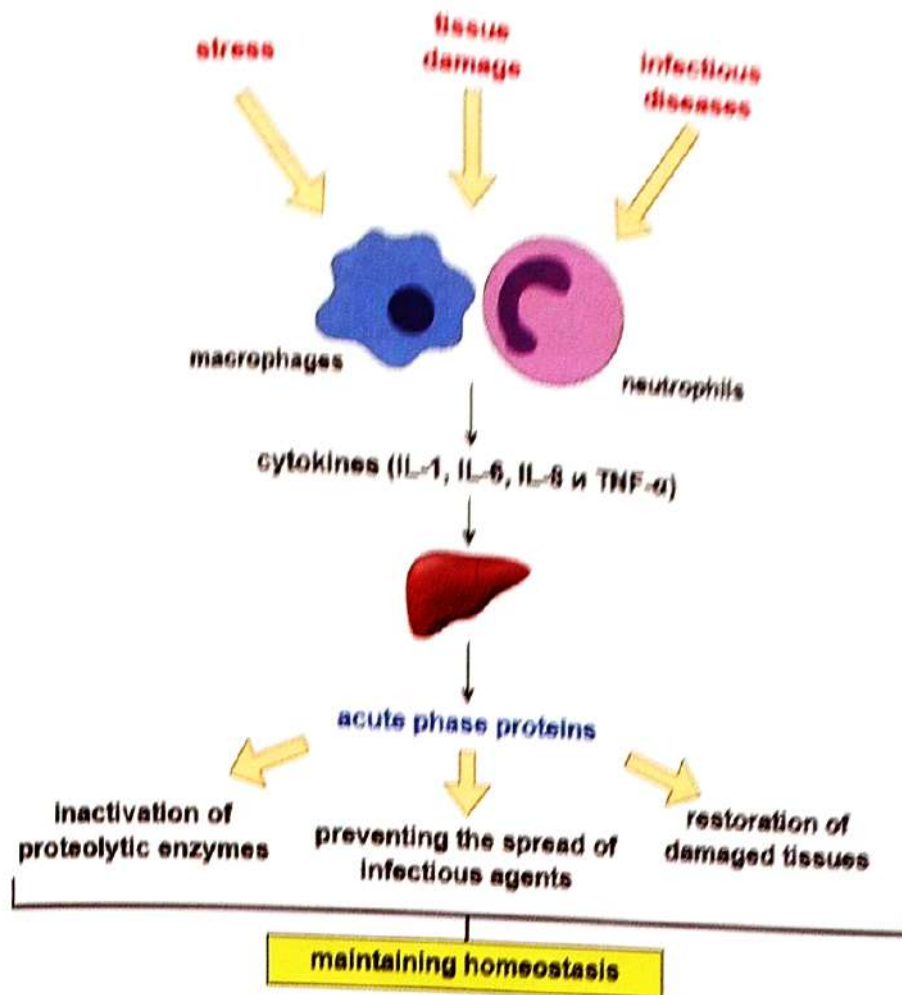


Fig. 15-5 Formation and action of acute phase proteins

Albumin

Albumin is the main protein in human plasma.

It (69 kDa) constitutes approximately 60% of the total plasma protein. 40% of albumin is found in plasma, while 60% is in the extracellular matrix. It is initially synthesized as a preproprotein. The synthesis of albumin is particularly suppressed in liver diseases and inflammation. Plasma levels in patients with liver diseases show a decreased albumin/globulin ratio. Albumin synthesis also decreases early in cases of impaired protein nutrition, such as in kwashiorkor. Mature albumin has three domains that perform different functions. It has an ellipsoidal shape, meaning it does not increase plasma viscosity as elongated molecules do, such as fibrinogen. The absence or drastic reduction of albumin levels in the blood is called **analbuminemia**. One cause is a **mutation** affecting **splicing**. It is believed that the levels of other plasma proteins increase to **compensate** for the absence of albumin.

Role:

- Due to its low weight and high concentration, albumin is responsible for 75-80% of the osmotic pressure of plasma.
- It transports various ligands: free fatty acids, calcium, certain steroid hormones, bilirubin, and part of the plasma tryptophan. Additionally, it plays a role in the transport of copper and many drugs, including sulfonamides, penicillin, dicoumarol, and aspirin.

C-reactive protein (CRP) (Fig. 15-6)

It is named because it reacts with the C-polysaccharide of pneumococci. CRP is a pentameric protein composed of five subunits arranged in cyclic symmetry. CRP is used in clinical practice to detect inflammatory processes! A decrease in plasma CRP concentration indicates a response to infection treatment.

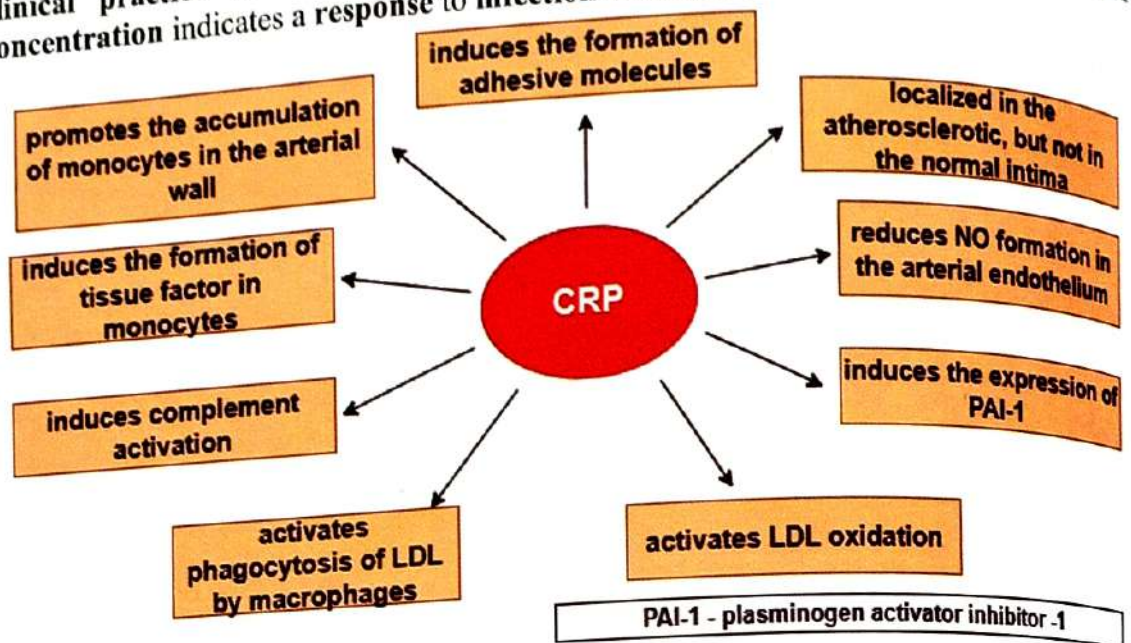


Fig. 15-6 Action of CRP

Haptoglobin

Haptoglobin (Hp) is synthesized in the liver and is a plasma glycoprotein that transports hemoglobin (Hb) in a stable non-covalent complex (Hb-Hp). Approximately 10% of Hb is degraded intravascularly every day. The remaining 90% of Hb is in old erythrocytes, which are degraded in the cells of the reticuloendothelial system (RES). Free Hb passes through the glomeruli of the kidneys, enters the tubules, and may precipitate there (as can occur after massive hemotransfusion) if the binding capacity of Hp is vastly exceeded. However, the Hb-Hp complex is too large to pass through the glomeruli. Therefore, Hp's function is to prevent the loss of free Hb in the kidneys. This preserves the vital iron ion found in Hb, which would otherwise be lost to the body.

Hb-Hp complexes are ligands for the CD163 receptor. Hemopexin-heme complexes are recognized by CD91 receptors. Endocytosis of the ligand leads to degradation in lysosomes, while the receptor is recycled back to the plasma membrane (Fig. 15-7).

The levels of Hp in human plasma vary and have diagnostic significance. Low Hp levels are observed in patients with hemolytic anemia. This is explained by the fact that the half-life of Hp is approximately 5 days, while the half-life of the Hb-Hp complex is about 90 minutes and is removed from plasma more rapidly by hepatocytes. Thus, when Hp is bound to Hb, it is cleared from plasma approximately 80 times faster than normal. Hp is an acute-phase protein, and its plasma levels increase in various inflammatory conditions.

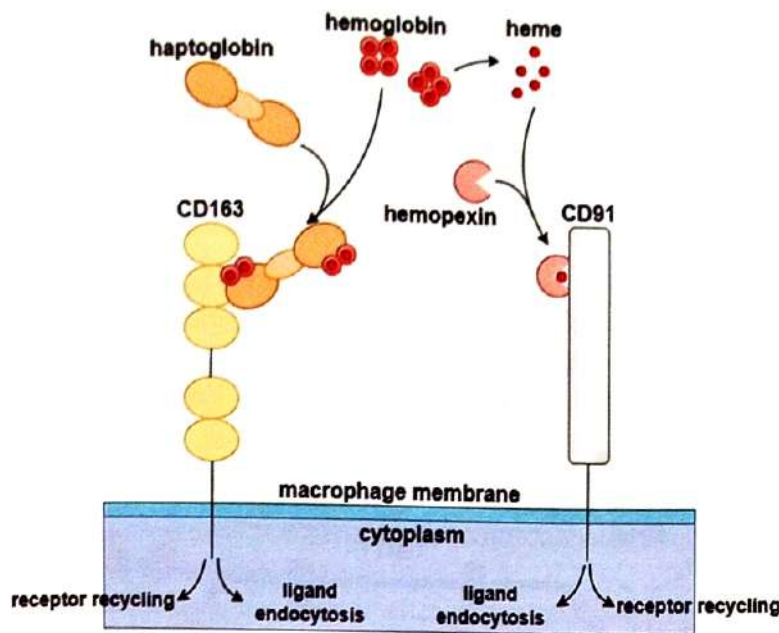


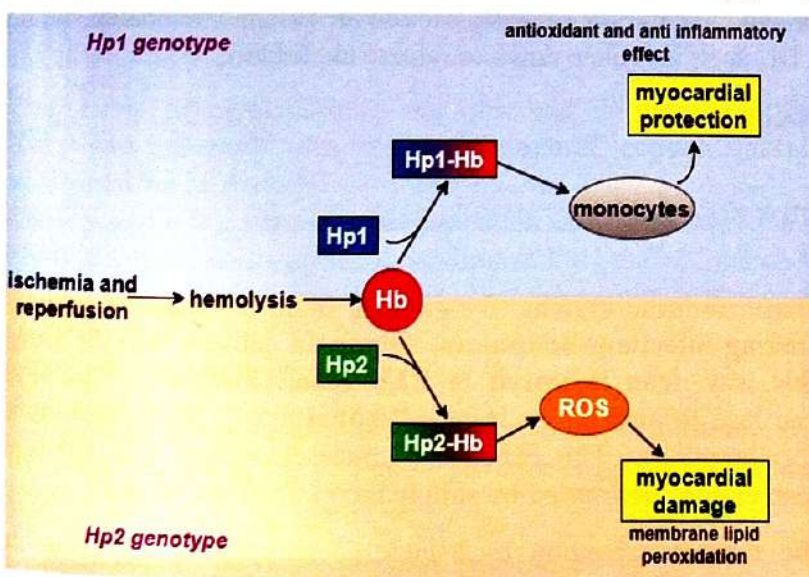
Fig. 15-7 CD163 pathway for uptake of Hb-Hp complexes and CD91 pathway for uptake of hemopexin-heme complexes

Hemopexin

Hemopexin is another protein (β -globulin) that transports free **heme**. **Albumin** binds a certain amount of **MetHb** (ferrihemoglobin) as **met-hem-albumin** and transports it to **hemopexin**.

Other causes of **hemolysis** with a decrease in **haptoglobin** levels include: **blood transfusion**, **artificial heart valve**, **erythrocyte deformation**, and **pathologically altered hemoglobin**, such as in **sickle cell anemia** and **thalassemias**.

During **ischemia/reperfusion** of the **myocardium** and **hemolysis**, hemoglobin is released. **Hp1-Hb complexes** are transported to the **liver** by **monocytes**, thereby protecting the **myocardium** from **oxidative stress**. However, the **Hp2 phenotype**, when bound to **hemoglobin**, exacerbates the **toxic effects** of **reactive oxygen species (ROS)**, which initiate



membrane lipid peroxidation and have a **pro-inflammatory effect** (Fig. 15-8).

Fig. 15-8 Involvement of Hp-Hb complexes in myocardial disorders

Transferrin

Transferrin (Tf) is a β -globulin. It is a **glycoprotein** synthesized in the **liver**. Tf plays a central role in the **transport of iron (2 moles of Fe^{3+} per molecule of Tf)** to locations where it is needed, i.e., from the **intestines to the bone marrow, liver, and other organs**.

Approximately **200 billion erythrocytes** are **catabolized** daily, releasing **25 mg of iron** into the body. **Free iron is toxic (via oxidative stress)**, but its binding to Tf **reduces** its potential toxicity. Tf binds to **transferrin receptors (TfR)** on cells and enters them via **receptor-mediated endocytosis** (Fig. 15-9, see Fig. 7-1 from Medical Biochemistry Part I).

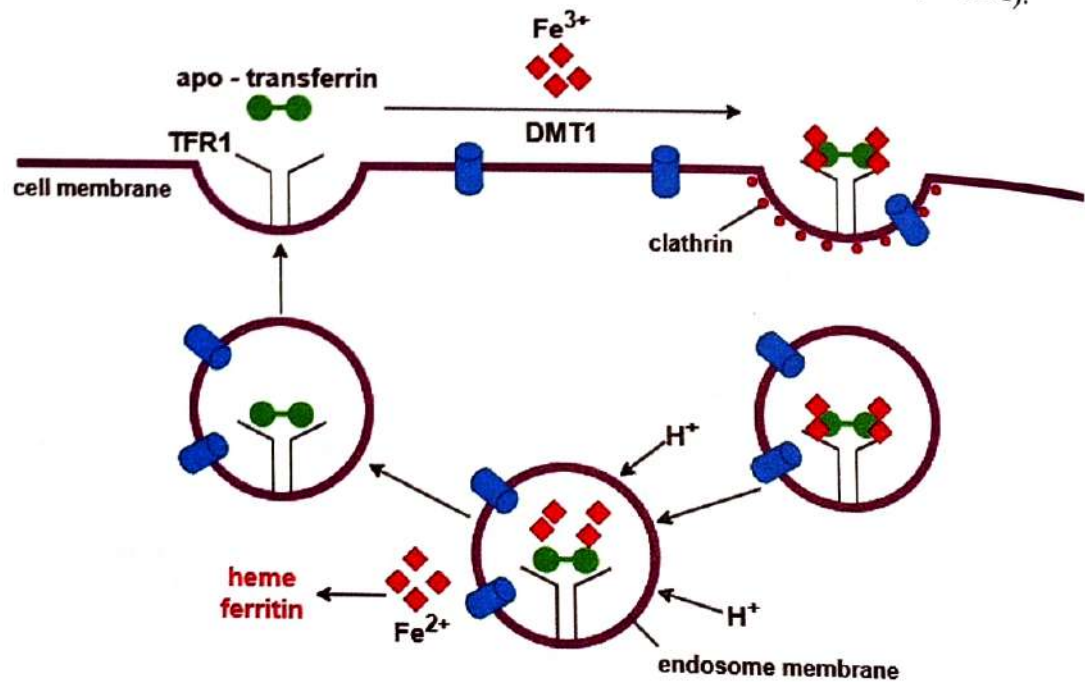


Fig. 15-9 Endocytosis of the iron, transferrin, and transferrin receptor complex

At an **acidic pH of 5.6** in the **lysosomes**, **iron dissociates** from **transferrin**. Unlike LDL, which is degraded in lysosomes along with its protein component, **apo-transferrin (apo-Tf) remains bound** to its receptor and the complex **recycles** back to the plasma membrane. While LDL receptors also recycle, LDL itself is broken down to release cholesterol.

Transferrin has three subgroups:

1. Serum transferrin
2. Lactotransferrin (lactoferrin)
3. Melanotransferrin

Transferrin is part of the **innate immune system**. By **binding iron**, it **hinders bacterial survival**. After binding iron during **infections or tumors**, transferrin delivers it to the **iron-storage protein ferritin**. This way, **iron is stored in RES cells**. However, in **chronic infections**, this mechanism can lead to **anemia**, as iron is "**sequestered**" from the blood, reducing its availability for erythropoiesis. This explains anemias associated with **chronic inflammatory processes**, which **cannot be treated** by simply increasing iron intake.

Atransferrinemia: A **genetic mutation** leading to a **lack of transferrin**, resulting in **hemosiderosis** in the heart and liver, which can lead to **heart and liver failure**. Treatment is done via **plasma infusion**.

Ferritin

Ferritin serves as an **iron storage depot**. It is an **acute-phase protein**. Ferritin contains approximately **23% iron** and **apoferritin** (the iron-free protein). It consists of **24 subunits** and holds **3,000–4,500 iron atoms**. Normally, ferritin levels in human plasma are **low**. However, in patients with **iron overload**, plasma ferritin levels increase significantly.

The **serum ferritin concentration** serves as an **indicator** of the **iron stores** in the body.

Hemosiderin is a **partially degraded form of ferritin**, still containing iron. It characterizes conditions associated with **iron excess** in the body. It can be detected through **tissue biopsy** and **staining with Prussian blue**.

Primary and secondary hemochromatosis

- **Primary hemochromatosis** is a **genetic disorder** characterized by an **excess of iron in tissues**, leading to **tissue damage**.
- **Secondary hemochromatosis** occurs after **repeated blood transfusions** (e.g., in the treatment of **iron-deficiency anemia**) or **excessive iron intake**.

Ceruloplasmin

Ceruloplasmin is an **α_2 -globulin** that functions as an **antioxidant**, a **copper-transporting protein**, and is involved in **iron homeostasis** (*ferroxidase activity*). It is primarily produced in the **liver** as an **acute-phase protein**, but other cells can also synthesize it, including **astrocytes**, **T- and B-lymphocytes**, **chondrocytes**, **macrophages**, **smooth muscle cells**, and others.

Other biological functions of ceruloplasmin:

1. Participates in LDL oxidation.
2. Erythrocytes possess receptors for ceruloplasmin.
3. Inhibits NOS in endothelial cells.
4. Induces aggregation of newly differentiated neurons in the nervous system.
5. Enhances IL-2-dependent cytotoxicity of T-lymphocytes and phagocytic activity of neutrophils and monocytes.

Low levels of ceruloplasmin are observed in **hepatolenticular degeneration** (**Wilson-Konovalov disease**) due to an **abnormal copper metabolism**, leading to **copper accumulation in tissues**.

Ceruloplasmin is a **plasma metalloprotein** that **binds 90–95% of plasma copper** and acts as a **ferroxidase**, catalyzing the **oxidation of Fe^{2+} to Fe^{3+}** , which is then bound by **transferrin**. During this oxidation, the **copper in ceruloplasmin transitions from Cu^{2+} to Cu^{1+}** .

Ceruloplasmin deficiency

Congenital ceruloplasmin deficiency (aceruloplasminemia) leads to **iron accumulation in most tissues**, indicating that *ceruloplasmin plays an essential role in iron homeostasis*. The following disorders are observed:

1. **Central Nervous System (CNS) Disorders**
 - The absence of ceruloplasmin in the serum is associated with iron accumulation in the brain and **neurodegeneration**.

- Ceruloplasmin induces iNOS expression in neuroglial cells and promotes NO release.
 - It enhances the expression of *pro-inflammatory molecules*, such as TNF- α , IL-1 β , COX-2, NADPH oxidase, and activates NF- κ B.
 - Regulates iron levels in the CNS, *preventing free-radical damage*.
2. **Liver and Reticuloendothelial System (RES) Disorders**
- Iron accumulation in the liver and spleen can be up to 36 times higher than normal.

α 2-Macroglobulin

α 2-Macroglobulin inactivates multiple **proteinases** (including **serine, cysteine, aspartic, and metalloproteinases**), functioning as a **pan-proteinase inhibitor (antiprotease)**.

α 2-Macroglobulins are **large plasma glycoproteins**, composed of **four identical subunits**, linked together by disulfide (-S-S-) bonds. Approximately **10% of plasma zinc** is transported by **α 2-macroglobulin**, while the remaining zinc is bound to **albumin**.

This protein is synthesized by various cell types, including **monocytes, hepatocytes, and astrocytes**.

The α -Macroglobulin family includes:

- α 2-Macroglobulin (α 2M)
- Complement components (C3, C4, and C5)
- Pregnancy zone protein (PZP)

Unique function of α 2-macroglobulin

Unlike other proteinase inhibitors, **α 2M does not directly inactivate proteinases**. Instead, it **restricts the access of large substrates to the active site of the proteinase**.

Since **cytokines** are involved in inflammatory processes, their binding to α 2-macroglobulin and subsequent removal helps **maintain a balanced and properly functioning immune system**. Once cytokine levels are restored to their optimal physiological balance, the immune system can protect the body and initiate the healing process. With the resumption of a normal inflammatory response, the regenerative processes of the immune system can function again.

α 2-macroglobulin and Alzheimer's Disease

α 2M protects against Alzheimer's disease by facilitating the degradation of A β (amyloid-beta) in neurons. To enter the neuron via the **LRP receptor** (from the ApoE family of LDL receptors), the **α 2M-A β complex must first bind to a proteinase**.

A variant of the α 2-macroglobulin gene, **α 2M-2**, is believed to be responsible for the formation of a **defective protein** involved in the pathogenesis of Alzheimer's disease. If α 2M-2 is defective, A β accumulates in the extracellular space, forming destructive **amyloid plaques** characteristic of Alzheimer's disease. **ApoE** may accelerate disease progression by **competing with the α 2M-2-A β complex for the LRP receptor**.

α 2-macroglobulin is also a coagulation inhibitor, as it **inhibits plasmin** (Fig. 15-10).

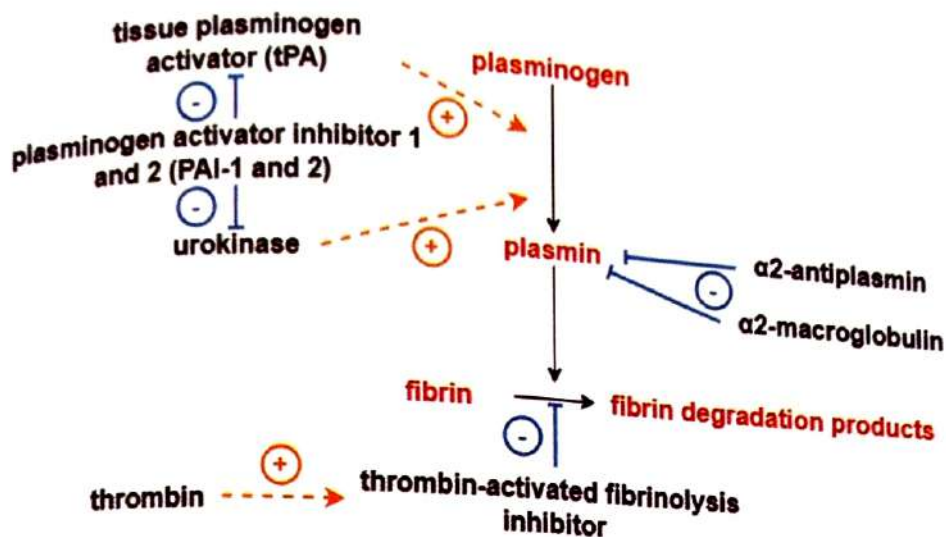


Fig. 15-10 The antiprotease α 2-macroglobulin is an inhibitor of blood clotting

α 1-Antitrypsin

α 1-antitrypsin (A1AT) is a **single-chain** protein containing **three oligosaccharide chains** and constitutes over **90%** of the α 1-fraction in plasma. It is synthesized in the **liver** and **macrophages** and serves as the **primary inhibitor of serine proteases** (serpin, Pi) in plasma. A1AT **inhibits** trypsin, elastase, and other proteases by forming complexes with them.

There are **three clinically relevant aspects** of α 1-antitrypsin:

1. Deficiency of α 1-antitrypsin is observed in **5% of emphysema** cases, where individuals with a ZZ genotype produce **lower** levels of α 1-antitrypsin. This leads to the **accumulation** of neutrophils and pulmonary inflammation. The lack of sufficient inhibitor allows neutrophil elastase (**trypsin**) to **degrade elastin**, leading to its increased breakdown. **Intravenous administration** of α 1-antitrypsin is used as a treatment for emphysema resulting from α 1-antitrypsin deficiency.

Elastin fibers form a dense network around pulmonary capillaries. Neutrophils, macrophages, and dendritic cells produce proteases, including **neutrophil elastase and matrix metalloproteinase-12 (MMP-12)**, as well as many other proteases identified in emphysema that degrade elastin, collagen, and fibronectin. Loss of alveolar scaffolding due to enzymatic degradation leads to **expansion of air spaces**. Additionally, elastin peptides exhibit chemotactic properties, attracting more inflammatory cells to the lungs, creating a vicious cycle of tissue destruction.

2. Another significant aspect is that **methionine at position 358 in α 1-antitrypsin** is critical for its **binding to proteases**. **Cigarette smoking** causes the **oxidation** of this methionine to **methionine sulfoxide**, rendering the affected molecule incapable of binding and neutralizing elastase.

3. Deficiency of α 1-antitrypsin is also associated with a **liver disorder** in which polymeric aggregates of mutant α 1-antitrypsin **accumulate** within the endoplasmic reticulum of hepatocytes. These polymers form through interactions between individual molecules in specific regions, leading to **hepatitis, which may progress to cirrhosis**.

Complement System

The complement system is a component of **innate immunity**, consisting of multiple plasma proteins that are activated during **infection** to target **opsonized antigens**, induce inflammatory responses, enhance **antibody-mediated** responses, and directly **attack** certain pathogens.

The activation of the complement **cascade** relies on the **cleavage of zymogens** (inactive enzymes), leading to the sequential activation of the next enzyme in the cascade. This chain reaction enables the immune system to **initiate and amplify** immune responses.

There are **three pathways** that can activate the complement system:

1. Classical pathway
2. Alternative pathway
3. Mannose-binding lectin (MBL) pathway

Deficiency or mutations in complement protein **C3** can disable the entire cascade. C3 mutations are rare and are inherited in an autosomal recessive manner. Individuals with C3 deficiency experience **frequent infections** in early childhood and have a high mortality rate.

Matrix Metalloproteinases (MMPs)

MMPs are a family of **25 Zn^{2+} -dependent neutral endopeptidases** that degrade **major extracellular matrix (ECM) components** and **basement membranes**.

MMPs participate in **various physiological processes**, including:

- Wound healing
- Angiogenesis
- Embryonic development
- Organ morphogenesis
- Leukocyte migration

On the other hand, **increased MMP expression and activity** play a key **pathogenetic role** in conditions characterized by **excessive ECM degradation**, such as:

- Rheumatoid arthritis
- Osteoarthritis
- Chronic ulcers
- Periodontitis
- Tumor invasion and metastasis

Specific MMP roles:

- **MMP-2** plays a role in **skeletal development, tissue repair, and tumorigenesis**.
- **MMP-7** stimulates **bone differentiation and ECM degradation**.
- **MMP-9** is involved in **bone remodeling**.
- **MMPs contribute to periodontitis progression**, with **MMP-8** being the most abundant in **saliva and periodontal tissues**.

While most MMPs are secretory proteins, certain types, known as membrane-type MMPs (MT-MMPs), are **anchored to the plasma membrane**.

The **activity of MMPs** is tightly controlled at multiple levels:

- Gene expression regulation

- Activation of latent zymogens
- Interaction with specific ECM proteins
- Inhibition by endogenous inhibitors, such as tissue inhibitors of matrix metalloproteinases (TIMPs)

TIMPs are a platform for developing "intelligent" drugs targeting MMP activity.

MMPs play a key role in **tumor metastasis and angiogenesis**, facilitating the spread of cancer cells and the **formation of new blood vessels**.

Serum Amyloid A

Serum amyloid A (SAA) proteins are a family of **apolipoproteins**, associated with **high-density lipoproteins (HDL)** in the plasma. Different **isoforms** of SAA are constantly expressed (**constitutive SAAs**) with varying levels or are produced in response to **inflammatory stimuli** (SAA is an **acute-phase protein**). These proteins are primarily produced in the **liver**. They perform several functions:

1. Transport of **cholesterol to the liver** for biliary secretion.
2. **Attraction** of immune cells to sites of inflammation.
3. Induction of enzymes that **degrade** the extracellular matrix.
4. Acute-phase serum amyloid A proteins (A-SAAs) are involved in several **chronic inflammatory diseases**, such as amyloidosis, atherosclerosis, and rheumatoid arthritis.

Amyloidosis

Amyloidosis is a disorder in which **insoluble proteins accumulate** in tissues and organs, impairing their function. The *heart, kidneys, liver, skin, lungs, and muscles* can be affected. Swelling in soft tissues can occur due to **fluid retention and amyloid infiltration** in the synovial membrane and surrounding tissues. A characteristic change is the **enlargement of the anterior shoulder region**.

Plasma Immunoglobulins

The **immune system** consists of two main types of **cells**:

1. **B-lymphocytes** - primarily produced in the **bone marrow**.
2. **T-lymphocytes** - originating from the **thymus**.

B-cells are responsible for producing **antibodies (immunoglobulins)**. **T-cells** are involved in **cell-mediated immune responses**, such as transplant rejection, hypersensitivity reactions, antitumor, and antiviral immunity.

Plasma immunoglobulins are synthesized by **plasmocytes**—specialized B-cell lineages that produce and secrete immunoglobulins into the plasma in response to various antigens.

All Ig molecules consist of **two identical light (L) chains and two identical heavy (H) chains**, forming a tetramer (L₂H₂) via **disulfide bridges**.

Humans have **five types of heavy chains: γ , α , μ , δ , and ϵ** . The type of heavy chain determines the Ig class, resulting in **five Ig classes: IgG, IgA, IgM, IgD, and IgE**.

Role of plasma proteins in inflammatory response mechanisms

Inflammation is a complex biological process in which white blood cells and chemical compounds **protect** against foreign substances, such as **bacteria, viruses, and certain chemicals**. This is a **defense mechanism** of the body to eliminate harmful substances and initiate tissue healing. As such, inflammation is part of the **regenerative process**. Without inflammation, wounds and infections would not heal, leading to progressive tissue destruction. **The goal is not to stop inflammation, but to restore normal inflammatory response.** In some cases, however, the immune system improperly initiates an inflammatory response even when **no** foreign substance is present. In such **autoimmune** conditions, the body's protective immune reactions cause damage to its own tissues. The biological processes of the immune system that maintain a normal inflammatory process are precisely and carefully regulated by **cytokines**—signaling proteins and glycoproteins involved in cellular communication.

The five characteristic **clinical signs of inflammation** are:

- Redness (rubor)
- Heat (calor)
- Swelling (tumor)
- Pain (dolor)
- Loss of function (functio laesa)

Excessive or **chronic inflammation** leads to an increase in **serum biomarkers of inflammation**, which are associated with **increased morbidity and mortality**.

Inflammation biomarkers:

- Increased erythrocyte sedimentation rate (ESR)
- Elevated C-reactive protein (CRP)
- Increased circulating immune complexes
- Increased cytokine production with disrupted Th1/Th2 balance
- Abnormal immunoglobulin (IgG, IgE, IgA, IgM) levels
- Enhanced fibrinogen activation leading to fibrin formation and fibrosis
- Increased amyloid production and deposition

Bacterial fragments stimulate the monocyte-macrophage system to produce cytokines. **Interleukin-6 (IL-6)** is the primary cytokine affecting **liver synthesis of acute-phase proteins**. It causes a mild increase in *ceruloplasmin*, *C3*, and *C4*. *Haptoglobin* and *fibrinogen* may increase 2-5 times. Plasma concentrations of *albumin* and *transferrin* are constitutively reduced, classifying them as "**negative**" **acute-phase proteins**. The two major positive acute-phase proteins are **CRP** and **serum amyloid A (SAA)**, which can increase over **1000 times**.

T-helper Cells (Th) and Immune Regulation

T-helper (Th) cells play a central role in **cell-mediated immunity**. **Antigen-presenting cells (APCs)** "present" antigens to Th. Th cells recognize specific epitopes, which are selected as target epitopes. Th cells assist B-cells in producing antibodies and also activate other immune cells.

The activating signals produced by Th cells are **cytokines**. Th cells are divided into two main subclasses based on their cytokine production:

- **Th1 cells** produce **pro-inflammatory cytokines** – IFN- γ , TNF- α , IL-2.
- **Th2 cells** produce **anti-inflammatory cytokines** – IL-4, IL-5, IL-10, IL-13.

Hemostasis (blood coagulation)

Hemostasis is a homeostatic, protective process, whose primary goal is to prevent blood loss and restore the integrity of the vascular wall.

Thrombosis

Thrombosis refers to the formation or presence of a **blood clot** in a blood vessel. The vessel can be any **vein or artery**, such as in deep vein thrombosis or coronary (arterial) thrombosis. The clot itself is called a **thrombus**. If the clot disintegrates and travels through circulation, this is known as **thromboembolism**.

In hemostasis, **initially, vasoconstriction** occurs to reduce blood flow. In small blood vessels, this can sometimes be enough to stop bleeding.

Hemostasis involves three main processes:

1. **Thrombogenesis** – formation of a thrombus (includes adhesion, activation, and aggregation of platelets (Tr)).
2. **Formation of a fibrin network** – coagulation of blood, carried out through autocatalytic reactions between plasma factors in the coagulation cascade, the final result of which is the conversion of fibrinogen into fibrin (Fig. 15-11).
3. **Fibrinolysis** – degradation of fibrin, which stabilizes the thrombus, and restoration of vessel integrity.

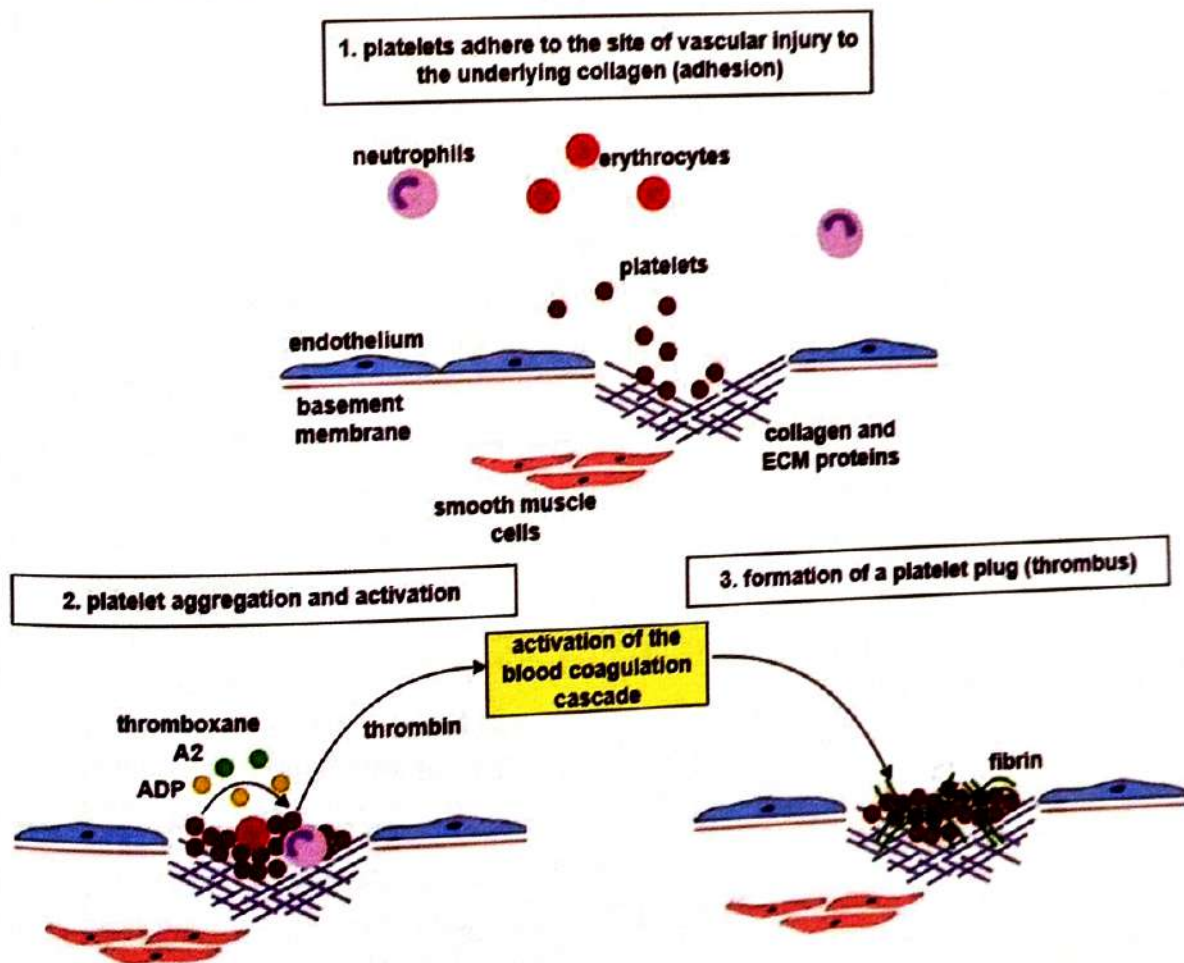


Fig. 15-11 Hemostasis

Three main factors participate in the hemostasis process:

- I. Endothelial cells
- II. Circulating blood cells
- III. Plasma proteins

Endothelial cells

1. Under normal conditions, they are **antithrombogenic** – their cell membrane lacks receptors for binding platelets (Tr) and leukocytes.
2. Their membrane contains **heparan sulfate proteoglycans**, which activate antithrombogenic molecules.
3. They synthesize **prostacyclin (PGI₂)**, the most potent **anti-aggregant**, as well as **NO** (via eNOS), leading to **arterial relaxation**.
4. Upon stimulation, endothelial cells express **phospholipids (phosphatidylserine)** and adhesion molecules, allowing binding of Tr and leukocytes.
5. They are the main source of **von Willebrand factor (VWF)**, which participates in **platelet adhesion** and interacts with subendothelial extracellular matrix (ECM) molecules.
6. Endothelial cells possess the enzyme **adenosine diphosphatase**, which hydrolyzes ADP, thereby weakening its platelet aggregation effect.

Circulating blood cells

Erythrocytes – release large amounts of **ADP, Ca²⁺** ions, and other metabolites affecting coagulation. Their ruptured membranes provide a **phospholipid (phosphatidylserine) surface**, which facilitates hemostasis reactions.

Leukocytes – adhere to damaged endothelium, secrete enzymes from their granules, and activate hemostatic proteins.

Platelets (Tr) – under normal conditions, they circulate **laminarily** in the bloodstream. Upon **vessel injury**, they move **turbulently**, leading to:

- **Platelet adhesion** – Tr binds to **collagen in the subendothelium via VWF**.
- **Platelet activation** – Tr secretes **TxA₂**, one of the most potent platelet aggregants, which also induces **vasoconstriction**. They release **ADP, Ca²⁺**, and **serotonin** (also a vasoconstrictor).
- **Platelet aggregation** – Tr adhere to each other in a **receptor-mediated manner via fibrinogen receptors (GPIIb-IIIa)**.

Platelet adhesion to subendothelial collagen and activation of platelet aggregation

Thrombin is the strongest platelet activator. It interacts with a **transmembrane receptor** on their surface called **PAR (protease-activated receptor)**. The interaction with PAR activates **PLC β** (phospholipase C beta).

PLC β degrades membrane phospholipids, generating **lipid mediators**:

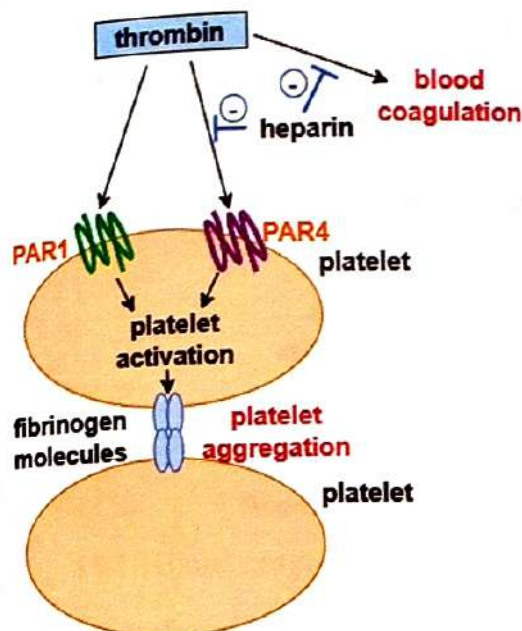
- **Inositol triphosphate (IP₃)**
- **Diacylglycerol (DAG)**

DAG activates **protein kinase C**, which phosphorylates and activates proteins, stimulating the release of granule components. IP₃ increases **intracellular Ca²⁺**, which binds to **calmodulin (CaM)**, activating the **myosin light chain kinase**. This leads to phosphorylation of myosin light chains and **interaction with actin**, causing **platelet shape change**.

All agents stimulating aggregation (ADP, collagen, thrombin, serotonin, TxA₂) modify the platelet surface, making it **capable of binding fibrinogen molecules**, which in turn link individual platelets into **aggregates (aggregation)**.

Thrombin receptors (Fig. 15-12)

Thrombin stimulates platelets via **PAR1** and **PAR4** (Protease-Activated Receptors), which belong to the **G protein-coupled receptor** family. Therefore, blocking both PAR1 and PAR4 is required for **effective prevention of thrombin-induced platelet activation**.



- PAR1 is the most studied thrombin receptor. The PAR1 antagonist Vorapaxar is used to prevent thrombosis in patients with a history of myocardial infarction.
- Although less studied, PAR4 is emerging as a promising target for antiplatelet therapy.

Fig. 15-12 Thrombin receptors

Receptors involved in platelet (Tr) function – Glycoprotein (GP) Iba and GPVI

GP_{Iba} and GPVI are the **primary adhesive/signaling receptors** on platelets (Tr), interacting with **VWF and collagen**, respectively. GPVI belongs to the **immunoglobulin (Ig) superfamily** and is expressed on the surface of Tr as a complex with the **Fc receptor, FcR γ** .

Plasma proteins in blood coagulation

Most **coagulation factors** are synthesized in an **inactive form by the liver**, while others are produced by **endothelial cells or other cell types**.

Coagulation factors are functionally divided into several groups:

1. **Enzymes** – most belong to the **serine protease group**, including factors II, VII, IX, X, XI, XII, plasmin, tPA (tissue plasminogen activator), kallikrein, etc. Factor XIII is a **transglutaminase**.
 2. **Cofactors** – also synthesized in **proforms**. They are not strictly “cofactors” but still **enhance reaction rates** – factors V, VIII, and **tissue factor (factor III)**.
 3. **Other factors** – fibrinogen (factor I), calcium ions (factor IV), phospholipids, and kininogens.
 4. **Regulatory proteins** – heparan sulfate, antithrombin III (activated in the presence of heparin), α 1-antitrypsin, protein C, protein S, thrombomodulin, α 2-antiplasmin, etc.
- After synthesis, many coagulation factors undergo various **post-translational modifications**, such as **carboxylation involving vitamin K**, making them **vitamin K-dependent factors** – factors II, VII, IX, X, proteins C and S.

Vitamin K is a cofactor of the enzyme *glutamate carboxylase*, which carboxylates glutamate (Glu) to γ -carboxyglutamate (Gla), enabling calcium ion (Ca^{2+} , factor IV) binding. Vitamin K is regenerated by *vitamin K epoxide reductase* (Fig. 15-13). This enzyme is inhibited by coumarin (indirect) anticoagulants.

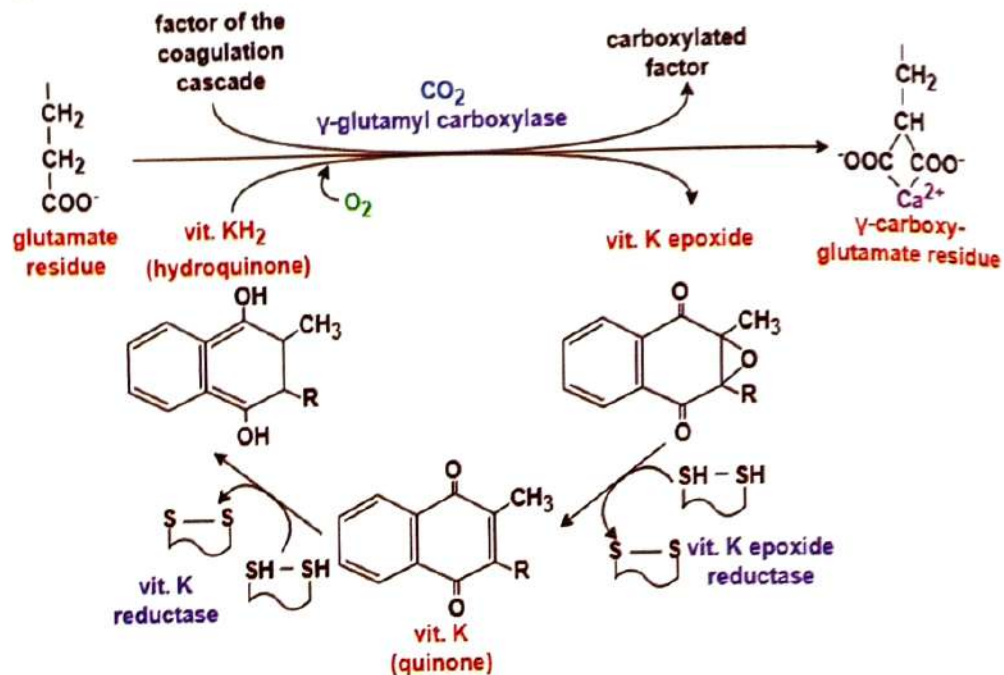


Fig. 15-13 γ -carboxylation of glutamate residues

Prevention and treatment of thrombosis

1. **Aspirin** – irreversibly acetylates and inhibits **COX-1**, preventing the production of TxA_2 in **platelets**. It also inhibits **COX-1** and **COX-2** in **endothelial cells**, reducing prostacyclin synthesis. However, unlike in platelets, cyclooxygenase regenerates within a few hours in endothelial cells.
2. **Platelet receptor blockers.**
3. **Direct and indirect anticoagulants.**

Coagulation pathways

Blood coagulation occurs in **three phases**:

- **Phase I** – formation of the prothrombin activation complex (Ca^{2+} , factors VIIIa, Va, IXa and Xa).
- **Phase II** – formation of **thrombin (factor IIa)**.
- **Phase III** – formation of **fibrin (factor Ia)**.

The **entire coagulation cascade** is a **stepwise series of reactions**, most of which are **proteolytic** and follow one another **until the final phase**.

Two **pathways** initiate coagulation:

- **Extrinsic pathway**
- **Intrinsic pathway**

Both involve **activation of proteolytic enzymes** (Fig. 15-14).

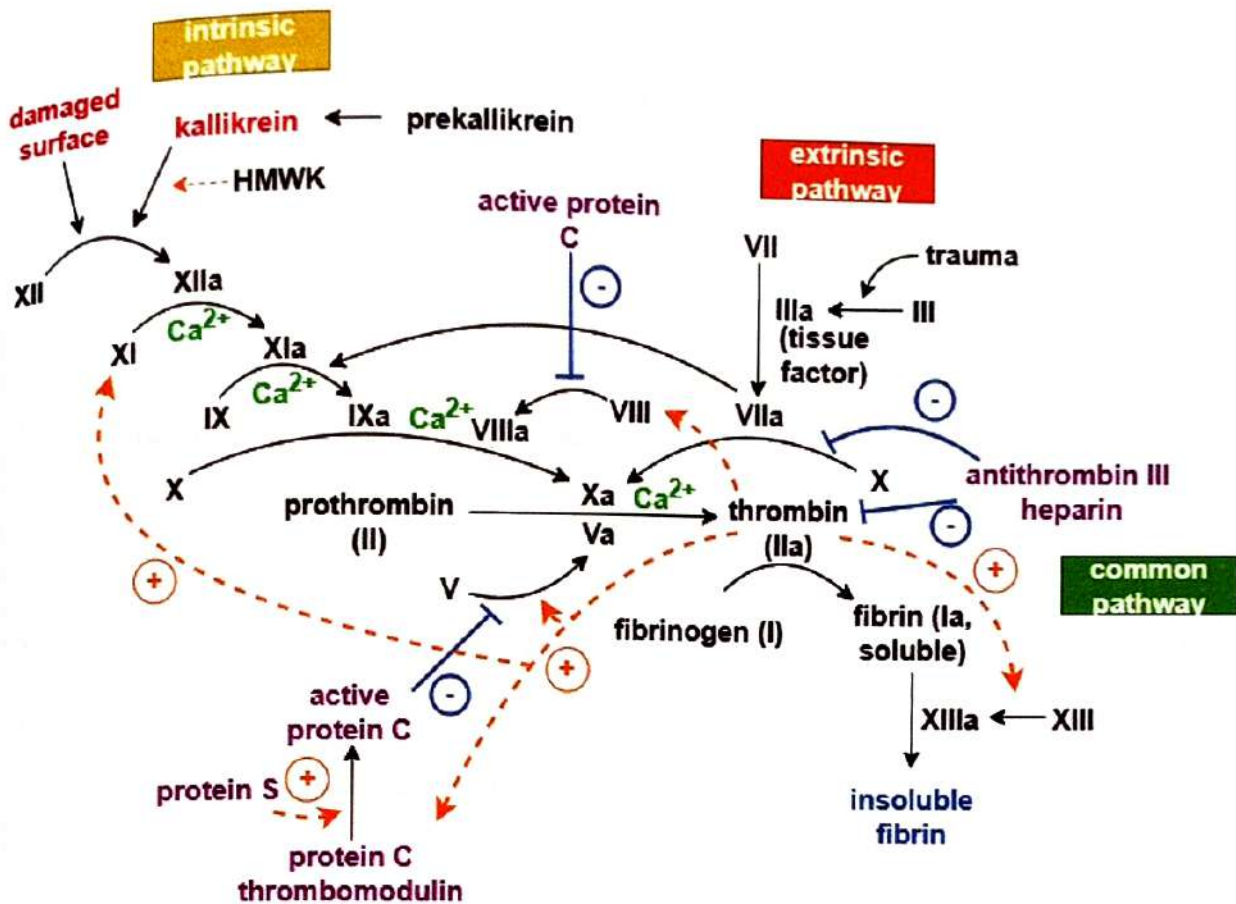


Fig. 15-14 Blood coagulation cascade

Intrinsic pathway

A key event is the activation of factor XII, prekallikrein, and high-molecular-weight kininogen (HMWK).

Prekallikrein is converted into kallikrein, which cleaves kinins such as bradykinin from HMWK. Factor XII binds to kallikrein and is converted into active factor XIIa, which in turn activates factor XI. Factor IX can be activated by either factor XIa or factor VIIa, undergoing two proteolytic cleavages to produce the active enzyme from a single polypeptide.

Extrinsic pathway

According to modern understanding, the extrinsic pathway is the primary mechanism for activating hemostasis, regardless of stimuli.

This pathway is initiated at sites of tissue injury, where endothelial cells express tissue factor (factor III). Tissue factor binds and activates factor VII to VIIa. Factor VIIa (a Gla-containing glycoprotein) activates factor X to Xa and factor IX to IXa. Factor X activation requires factor VIII, which functions as a cofactor.

Common pathway

- Factor Xa activates prothrombin (factor II) into thrombin (factor IIa).
- Factor V serves as a cofactor for factor Xa.
- Thrombin (factor IIa) converts fibrinogen into soluble fibrin monomers.

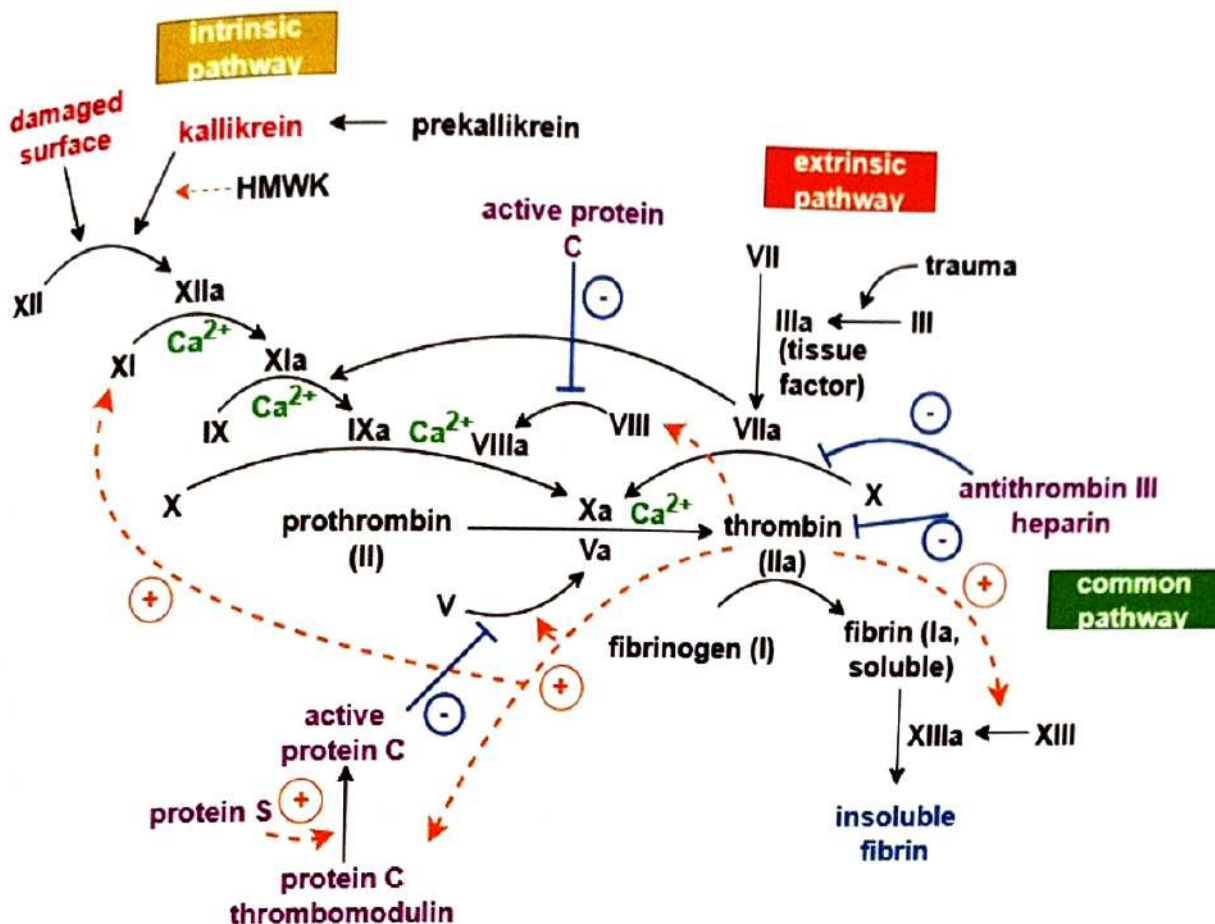


Fig. 15-14 Blood coagulation cascade

Intrinsic pathway

A key event is the activation of **factor XII**, **prekallikrein**, and **high-molecular-weight kininogen (HMWK)**.

Prekallikrein is converted into **kallikrein**, which cleaves **kinins** such as **bradykinin** from **HMWK**. **Factor XII** binds to **kallikrein** and is converted into active **factor XIIa**, which in turn activates **factor XI**. **Factor IX** can be activated by either **factor XIa** or **factor VIIa**, undergoing two proteolytic cleavages to produce the active enzyme from a single polypeptide.

Extrinsic pathway

According to modern understanding, the **extrinsic pathway** is the **primary mechanism** for activating **hemostasis**, regardless of stimuli.

This pathway is **initiated at sites of tissue injury**, where **endothelial cells** express **tissue factor (factor III)**. **Tissue factor** binds and activates **factor VII** to **VIIa**. **Factor VIIa** (a **Gla**-containing glycoprotein) activates **factor X** to **Xa** and **factor IX** to **IXa**. **Factor X** activation requires **factor VIII**, which functions as a cofactor.

Common pathway

- **Factor Xa** activates **prothrombin (factor II)** into **thrombin (factor IIa)**.
- **Factor V** serves as a cofactor for **factor Xa**.
- **Thrombin (factor IIa)** converts **fibrinogen** into **soluble fibrin** monomers.

Thrombin – a powerful enzyme (serine protease) with two major effects:

1. Acts on receptors on the surface of endothelial cells (PAR – protease-activated receptors).
2. Affects nearly all coagulation proteins, including:
 - Fibrinogen
 - Factor XIII
 - Most proenzymes of coagulation and fibrinolysis

Factor V and Factor VIII – similarities in structure and activation

- Both circulate as **inactive precursors** and are activated through **limited proteolysis** by thrombin or factor X.
- Factor VIII deficiency leads to **Hemophilia A (Classic Hemophilia)**, the most common form of hemophilia.
- Factor IX deficiency leads to **Hemophilia B**.
- Symptoms in both types are nearly identical, characterized by **prolonged bleeding, most commonly into joints**.
- Hemophilia is one of the **hereditary diseases** found in European aristocratic families.
- In the 19th and 20th centuries, hemophilia was present in most royal families in Europe, likely originating from Queen Victoria due to a spontaneous mutation.

Prothrombin activation into thrombin (Fig. 15-15)

Prothrombin, initially a **single-chain** polypeptide with 10 Glu (γ -carboxyglutamate) residues at its N-terminus, is converted into a **two-chain** form (A and B chains linked by S-S bonds).

The **positively charged Ca^{2+} on thrombin binds to the negatively charged surface** of the cell membrane.

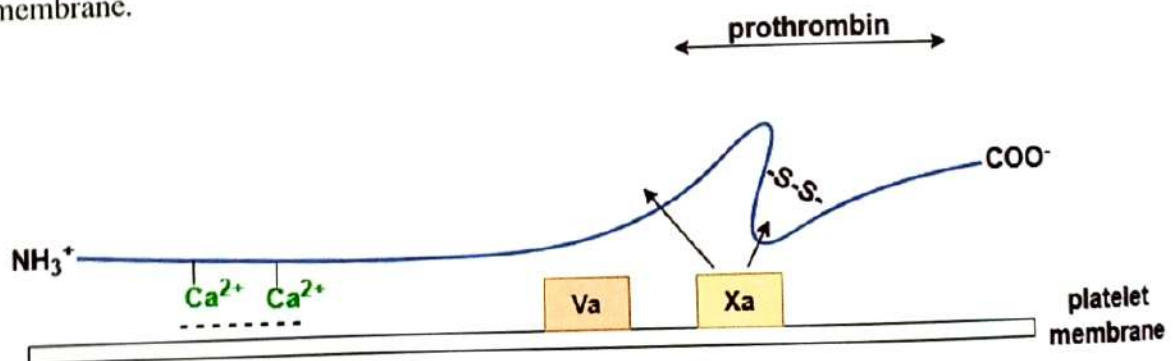


Fig. 15-15 Activation of prothrombin to thrombin

To make fibrin insoluble, thrombin activates **factor XIII (fibrin-stabilizing factor)**, which is found not only in blood but also in **connective tissue** (see Fig. 16-6).

- Factor XIII, also known as **transglutaminase**, links the γ -carboxyl groups of glutamine with the ϵ -amino groups of lysine through **covalent isopeptide bonds** (Fig. 15-16).
- This **cross-links fibrin strands**, stabilizing the **thrombus to an insoluble one**.

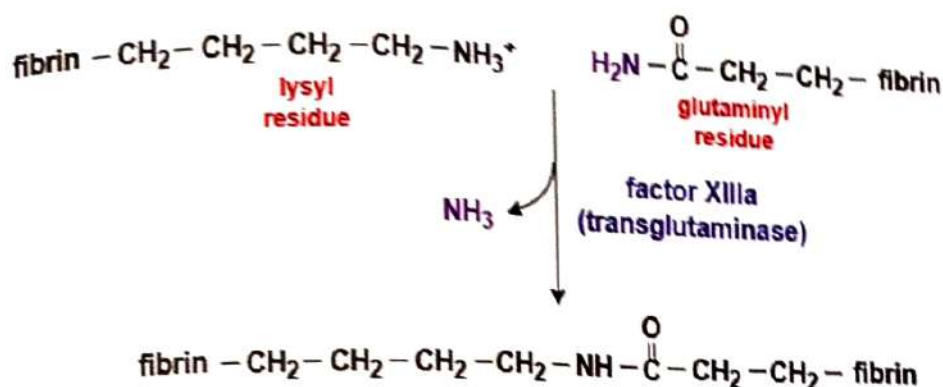


Fig. 15-16 Action of transglutaminase

Fibrinolysis (See Fig. 15-10)

A key event is the **activation of plasminogen** (an acute-phase protein) by tissue plasminogen activator (tPA) into plasmin.

- tPA exists in different isoforms – in the kidneys, it is called **urokinase (uPA)**.
- These activators are initially inactive and are activated by thrombin.
- **Serpins** (serine protease inhibitors), including **plasminogen activator inhibitor-1 (PAI-1)** and **plasminogen activator inhibitor-2 (PAI-2)**, irreversibly inhibit tPA and uPA.

Fibrinogen (Fig. 15-17)

- Composed of three polypeptide chains (α , β , γ) linked by numerous disulfide bonds.
- Fibrinogen is an **acute-phase protein**.
- Thrombin cleaves the α and β chains, producing **fibrinopeptides A and B** and forming monomers that spontaneously polymerize into soluble fibrin.

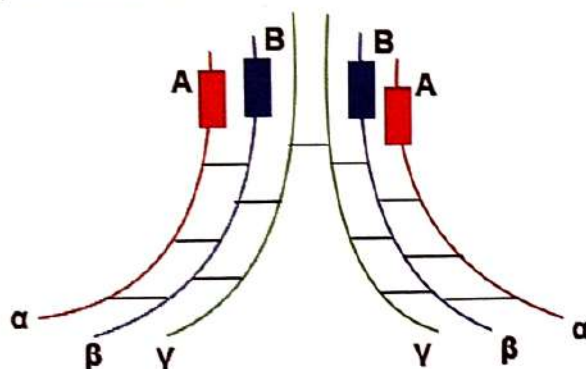


Fig. 15-17 Structure of fibrinogen

Regulation of hemostasis

Nearly all coagulation enzymes have circulating **inhibitors**.

The key thrombin inhibitor is **antithrombin III**, which requires **heparin** to bind thrombin.

- **Direct anticoagulants** act here.

Antithrombin III also inhibits other proteases, including XIIa, XIa, IXa, Xa, and VIIa. Factor V and Factor VIII are inhibited by **protein C**, which is activated by **protein S**. The key inhibitors of plasmin are **α 2-antiplasmin** and **α 2-macroglobulin** (see Fig. 15-10).

16. Mechanisms of Cell Adhesion

Adhesion is a vital property of cells. It provides a stable environment for cell growth and differentiation and allows cells to migrate. The adhesion of cells to each other, their extracellular matrix (ECM), and endothelial surfaces is mediated by various membrane proteins, collectively known as **cell adhesion molecules (CAMs)**.

Cell adhesion molecules

CAMs on the cell surface play a role in numerous **cellular processes**:

- Cell growth
- Cell differentiation
- Embryogenesis – CAMs guide cells around the embryo to grow and form the placenta
- Transmigration of immune cells and immune response
- Tumor cell metastasis – The lack of cell adhesion allows cancer cells to spread more easily from one part of the body to another
- Transmitting information from the ECM to the cell

Some diseases, such as **arthritis**, result from poor cell adhesion. This can occur when white blood cells are retained due to defective adhesion molecules. Measuring CAMs in serum or plasma allows their use as **markers of early endothelial dysfunction**. There are four major families of CAMs: Immunoglobulin Superfamily (IgSF), Integrins, Cadherins, and Selectins.

Immunoglobulin Superfamily (IgSF)

Role: IgSF participate in **cell recognition, binding, and adhesion**.

Structure (Fig. 16-1): Members of this family share structural characteristics with **immunoglobulins** (also known as **antibodies**). They are **transmembrane proteins** composed of an extracellular part – an **immunoglobulin domain** (made up of a series of repeating **IgG-like amino acid residues**), a **transmembrane region**, and a **short cytoplasmic segment**.

Types: Intercellular adhesion molecule 1 and 2 (**ICAM-1 and ICAM-2**), vascular cell adhesion molecule 1 (**VCAM-1**), expressed on endothelial cells; **ICAM-3** – on leukocytes; **ICAM-4** – on erythroid precursors; **ICAM-5** – in nervous tissue; platelet-endothelial cell adhesion molecule-1 (**PECAM-1**); junctional adhesion molecules (**JAMs**), located on the endothelium.

The **immunoglobulin-like GPVI** on platelets activates collagen binding when blood vessels are damaged.

The fusion of sperm with the egg during fertilization requires **IZUMO1** (Izumo sperm-egg fusion protein 1), another IgSF member.

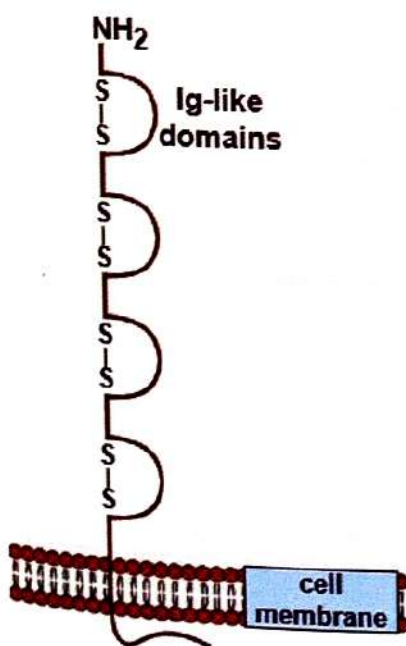


Fig. 16-1 Immunoglobulin superfamily

Integrins

Role: These are membrane receptors that play a crucial role in **signal transduction**. They mediate **cell-cell interactions and cell-ECM interactions**. Growth factors bind to membrane receptors and initiate cellular signaling, stimulating integrin expression. Integrins activate cell survival, migration, invasion, and tumor cell metastasis.

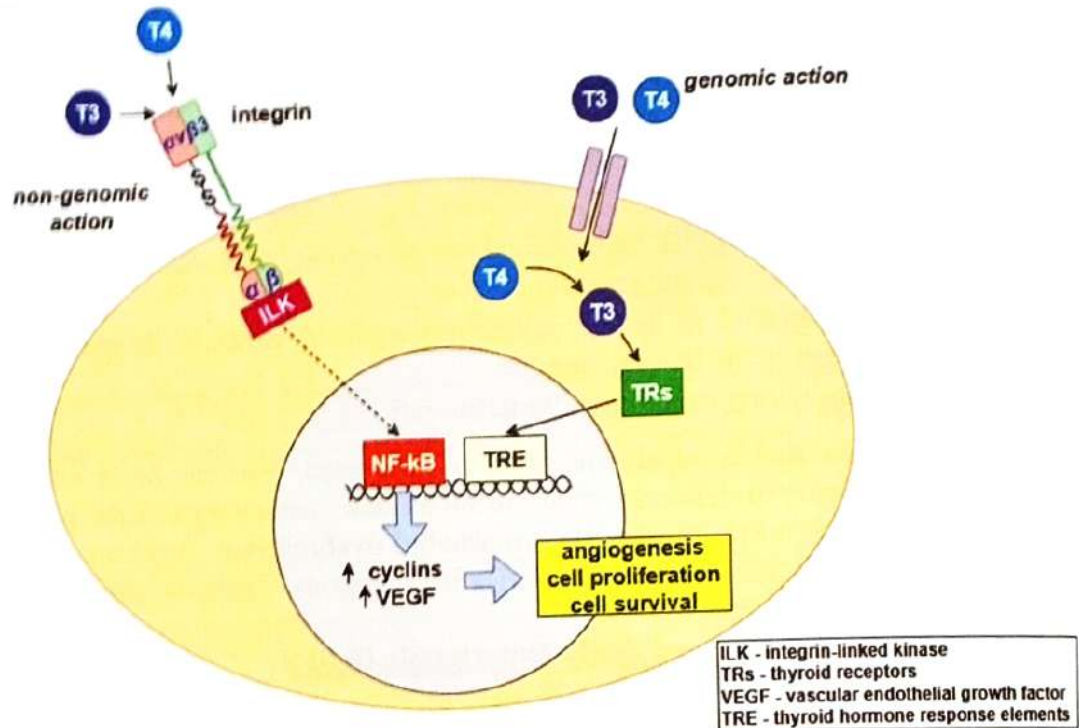


Fig. 16-2 Nongenomic action of thyroid hormones through the membrane receptor integrin $\alpha v \beta 3$

Integrin $\alpha v \beta 3$ is the membrane receptor involved in the non-genomic action of **thyroid hormones** (Fig. 16-2). This includes the activation of **NF- κ B** and the production of **angiogenic factors** such as *VEGF*, *cell proliferation*, *survival*, and *angiogenesis*.

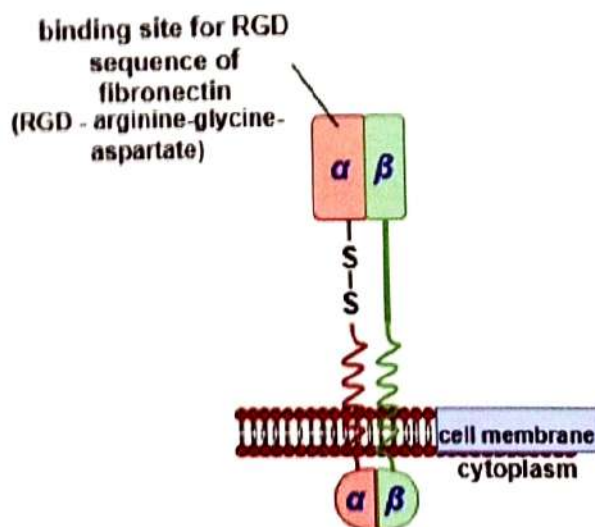


Fig. 16-3 Integrins

Structure: They are **$\alpha \beta$ heterodimeric complexes** (non-covalently linked membrane glycoprotein α and β subunits) (Fig. 16-3). Cytoplasmic domains bind to **adapter** proteins (e.g., α -actinin, talin, filamin) that connect integrins to cytoskeletal elements, such as **actin filaments**.

Types: Different **β subunits** define integrin types:

- **$\beta 1$ integrins** – VLA family (Very Late Antigen)
- **$\beta 2$ integrins** – LFA-1 family (Leukocyte Function Associated molecule-1)
- **$\beta 3$ integrins** – cytoadhesins, etc.

Cadherins

Role: Cadherins participate in **cell-cell contacts**, as well as in embryonic development and tissue organization. The tissue-specific expression of cadherin molecules is necessary for **cell sorting and recognition** during embryogenesis and for maintaining tissue architecture.

Structure: Their functional unit is a **dimer**. Cadherins contain **β -chains** with five characteristic repeats, arranged in two chains, forming a "sandwich" structure held together by **calcium ions** (Fig. 16-4). **Homophilic binding** between two cells occurs through identical terminal cadherin molecules. When calcium ions are bound, the extracellular domain has a **rigid, rod-like structure**. The adhesive activity of cadherins depends on their intracellular domains interacting with cytoplasmic proteins, such as **catenins**.

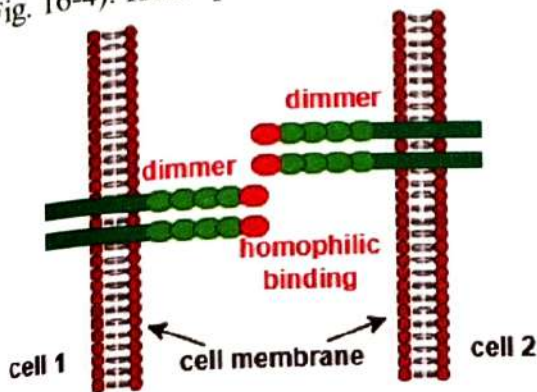


Fig. 16-4 Cadherins

Types: The **cadherin family** includes **neural (N)**, **placental (P)**, **epithelial (E)**, **cardiac (T)**, **brain (BR)**, **muscle (M)**, and many others. There are also **desmosomal cadherins** and **protocadherins**.

Selectins

Role: They support the **mobilization and adhesion of leukocytes** to the damaged endothelium, as well as their **transendothelial migration**, together with CAMs from the Ig-family. Selectins play an important role in the **early steps of leukocyte trafficking**. They mediate the **rolling** of leukocytes, while integrins and immunoglobulins enable firm adhesion and migration. The rolling mechanism is the most notable property of selectins. Selectin-binding sites are present on leukocytes, but **healthy endothelial cells do not express selectins**. However, in cases of *vascular damage or inflammation*, due to the release of cytokines, there is increased expression of adhesive molecules, leading to *enhanced adhesion and migration* of inflammatory cells across the vascular wall. The activation of endothelial cells leads to selectin expression, followed by interactions with their ligands on the leukocyte membrane. For example, upon leukocyte activation, their integrins ($\alpha L\beta 2$) bind to IgSF glycoproteins like **ICAM-1** and **VCAM-1**, allowing firm adhesion. The increased affinity of integrins is triggered by chemokines, bacterial peptides, platelet-activating factor (PAF), and leukotriene B₄. The **migration of leukocytes** is a key mechanism in the pathogenesis of **inflammatory diseases**, as well as in the regulation of hematopoiesis and hemostasis.

Biological role of CAMs in leukocyte transendothelial migration

Leukocyte transendothelial migration is an example of the diverse functions of adhesive molecules. This type of leukocyte trafficking consists of four stages (Fig. 16-5):

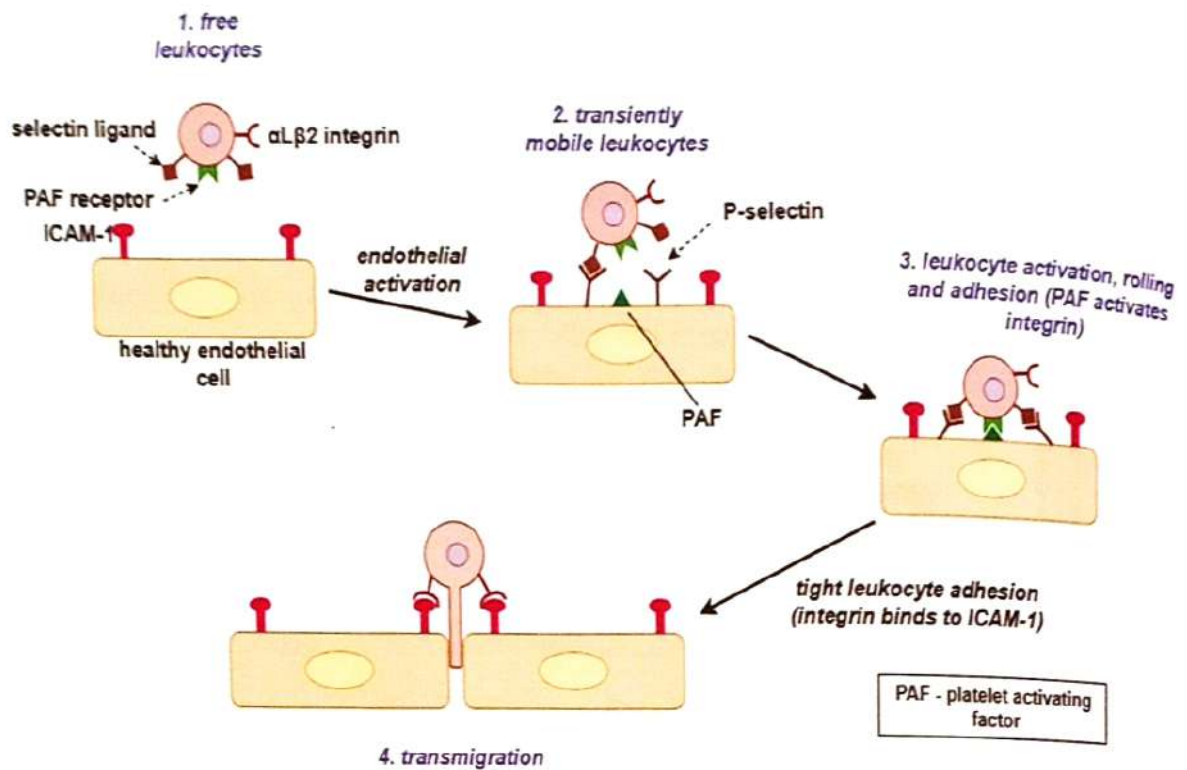


Fig. 16-5 Transendothelial migration of leukocytes (rolling, adhesion, tight-fitting, and migration)

The **first stage** is **rolling**, where circulating leukocytes move along the surface of endothelial cells. This step is mediated by **selectins**.

The **second stage** involves **triggering** or activation of cell surface adhesive molecules, specifically **integrins**. This can be influenced by contact with specific ECM proteins, inflammatory cytokines, or chemokines.

The **third stage** involves **firm adhesion**, where leukocytes attach tightly to the endothelial cell. **Integrins** and their ligands play a critical role in this step.

The **fourth stage** is **transmigration (crossing the membrane)** of leukocytes through adjacent endothelial cells, in a process called **diapedesis**. This step allows leukocytes to enter the subendothelial space. **PECAM-1** (platelet endothelial cell adhesion molecule-1) plays a key role in this step.

Transendothelial migration demonstrates the cooperation between leukocytes and endothelial CAMs. Such a complex process can be **disrupted** at different **stages**, each **regulated** by **specific adhesive molecules**.

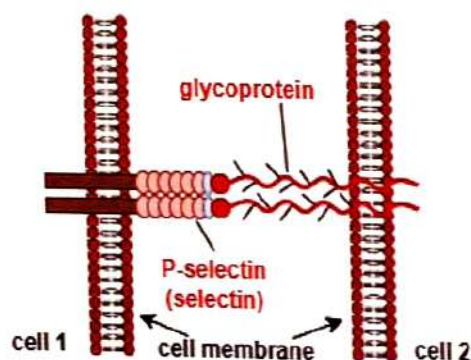


Fig. 16-6 Selectins

Structure: Selectins are a family of glycoproteins, whose activity depends on **divalent ions** (Fig. 16-6).

The **extracellular domain** of each selectin consists of a carbohydrate recognition motif, an epidermal growth factor-like motif (EGF-like motif), and a variable number of short, repeating domains, related to complement-regulatory proteins (CRP). Beyond the **transmembrane** region, each selectin has a short **cytosolic** region.

Types (Table 16-1):

Table 16-1 Types of selectins and their function

selectins	expression	function
L-selectin (leukocyte)	leukocytes (constant expression)	rolling
E-selectin (endothelium)	endothelial cells (synthesis in response to IL-1 and TNF)	capture, rolling, adhesion of Neu, Mo, Eo, T-Ly
P-selectin (platelet)	platelets, endothelial cells (synthesis upon stimulation by thrombin and histamine)	interaction between platelets and endothelium, also binds leukocytes upon stimulation by TNF, γ INF

Defects in cell adhesion molecules

Leukocyte adhesion deficiency syndrome (LAD) is associated with a cascade of adhesion defects.

- LAD I is linked to mutations in β 2-integrin.
- LAD II results from a defect in selectins.

CAMs also play a role in the formation of the **blood-brain barrier** and facilitate the **passage** of immune cells through it. **Selectins and integrins** are the most important adhesive molecules in this process. These molecules can also be exploited by pathogenic microorganisms to evade the immune system.

Cytoskeleton and cell adhesion

The cytoskeleton consists of dynamic filamentous structures (filaments). There are **three main types** of filaments: *actin filaments*, *intermediate filaments*, and *microtubules*. The cytoskeleton is not only a **structural** component but also participates in various cellular processes such as **transport**, **mitosis**, **secretion**, and **the formation of cellular extensions**. Cell adhesion provides **anchoring** points between epithelial cells to ensure proper integrity and resistance to mechanical stress on epithelial layers. There are different types of cell adhesion, mediated by specifically organized structures, namely: *desmosomes*, *hemidesmosomes*, *adherens junctions*, *tight junctions*, *gap junctions*, and *focal adhesions*. The association of adhesive structures with the cytoskeleton is necessary to stabilize cell-cell and cell-matrix adhesions and to integrate cell contacts with morphological changes typical for epithelial cells.

Actin filaments

Actin filaments are composed of **two chains** of polymerized actin protein. The diameter of the filaments is **5-9 nm**. Actin filaments are dispersed throughout the cell, but they are mainly concentrated just **beneath the plasma membrane**. They have a special role in the regulation of cell surface shape, forming *lamellipodia*, *filopodia*, and *microvilli*. They also participate in *clathrin-mediated endocytosis*, *phagocytosis*, *intercellular contacts*, and *neuronal synapses*. The formation of these structures is regulated by **Rho-GTP-binding proteins**, which belong to

the **Ras** protein superfamily. The ability of a cell to move is mainly due to actin filaments in complexes with **myosin** proteins. The actin monomer can exchange the bound **ADP** for **ATP**, which determines a different conformation of actin. **Globular G-actin** binds ATP (Fig. 16-7). It then forms stable **di- or trimers**, and finally, the filaments **elongate** by adding monomers. The hydrolysis of ATP to ADP leads to a distinction between the **fast-growing (+) end** and the **slow-growing or dissociating (-) end** of **F-actin (filamentous actin)**. F-actin can hydrolyze its bound $\text{ATP} \rightarrow \text{ADP} + \text{P}_i$, releasing P_i .

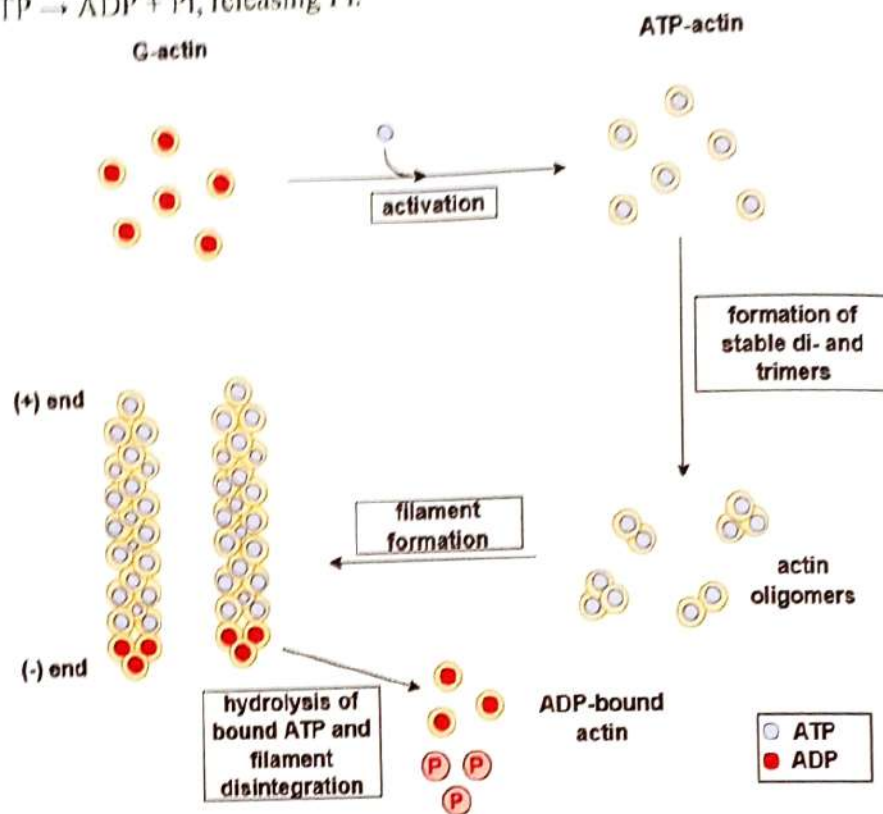
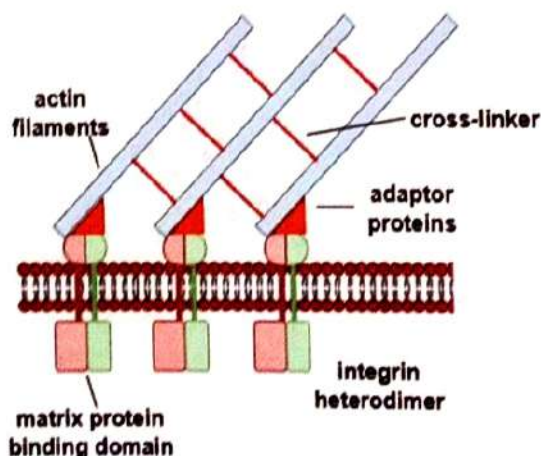


Fig. 16-7 Formation of actin filaments

ADP/ATP exchange: G-actin can release ADP and bind ATP, which is usually at higher concentrations than ADP in the cytosol. Actin filaments are polarized. All actin monomers are oriented with their **clefts** towards one end of the filament, called the **minus end**. Actin monomers spiral around the filament axis, forming a structure **similar to a double helix**. **Capping proteins** bind to the ends of actin filaments. Different capping proteins can stabilize an actin filament or promote its disassembly. They play a **role** in determining the length of filaments.



In **focal adhesions**, **actin** filaments attach through **adaptor** proteins to plasma membrane **integrins** (Fig. 16-8). Adaptor proteins that link actin filaments to the cytosolic domains of integrins are **α -actinin** and **talins**. A cell is **firmly attached** to the extracellular matrix when its extracellular integrin domains are connected to matrix proteins.

Fig. 16-8 Focal adhesions

Intermediate filaments

Intermediate filaments have a **diameter of 10 nm**. They are **divided into six types**:

1. **Nuclear lamins** – limit the deformability of the nucleus. Cancer cells with more deformable nuclei have a higher migration rate through small constrictions. Highly metastatic breast cancer cells express low levels of lamin A/C, leading to more flexible nuclei and enhanced migration through narrow spaces.
2. **Vimentin family (vimentin, desmin, and glial fibrillary acidic protein)**;
3. **Type I and Type II Keratins** (epithelial cells, hair, nails);
4. **Neurofilament proteins** (NF-L, NF-M, NF-H, α -internexin, synemin);
5. **Nestin** (expressed in neural stem cells).

Intermediate filaments are more resistant to **mechanical stress** compared to microtubules or actin filaments. They are highly **stretchable** and can be elongated by 240%-300% before breaking. They exhibit a strain-dependent stiffening response, which is often compared to a **seatbelt**. Mutations in genes for intermediate filaments are linked to various diseases involving defects in tissue or cell maturation. **Mutations in keratin** are associated with skin **blistering** and hypertrophy, conditions in which keratinocytes fail to withstand mechanical stress.

Structure: Intermediate filaments are made up of protein **monomers**, which spiral to form a **dimer**. Two dimers form a **tetramer**. **Eight tetramers** associate to form an intermediate filament with a diameter of **10 nm**.

Microtubules

Microtubules are the largest cytoskeletal components, involved in intracellular **transport (cell signaling)**, **cell migration/trafficking**, **cell division**, and **proliferation**. Microtubules control **intracellular rearrangements and changes** in cell morphology. They form the core components of **centrosomes and centrioles**, which are important for mitosis and the primary structures of **cilia**. Understanding the cellular structure and function of microtubules is essential for gaining deeper knowledge of processes such as *morphogenesis*, *wound healing*, *neurogenesis*, and *immune response*, as well as abnormal processes like *metastasis* and *angiogenesis* associated with tumors and neurodegenerative diseases.

Structure: Microtubules are composed of **tubulin heterodimeric** subunits. Each subunit consists of **α - and β -tubulin** proteins. The subunits attach to each other to form a **protofilament**. **Thirteen protofilaments** associate to form a tubular filament with a **25 nm diameter**. Microtubules originate from a **microtubule-organizing center or centrosome**. They are highly dynamic structures.

Tau (Tubulin Associated Unit) is an important protein that **regulates microtubules**. It is predominantly expressed in axons and **controls axonal transport**. Dysfunction in the Tau-microtubule pair is linked to numerous neurodegenerative diseases, commonly known as *Tauopathies*, including **Alzheimer's disease** and **Huntington's disease**. This group of neurodegenerative diseases is characterized by the **accumulation of abnormal Tau protein in the human brain** (Fig. 16-9).

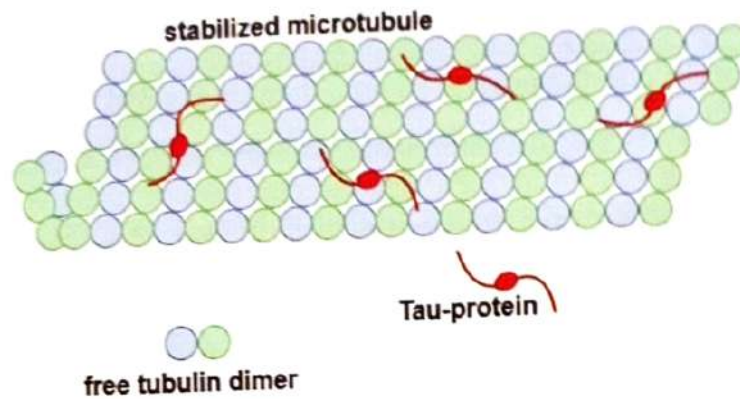


Fig. 16-9 Tau-protein

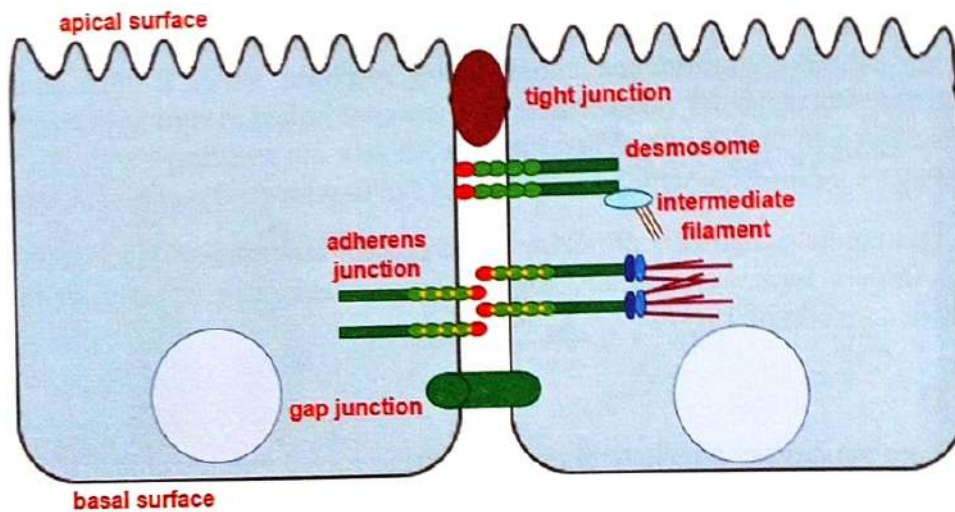


Fig. 16-10 Intercellular contacts

Intercellular Contacts (Fig. 16-10)

Intercellular contacts are classified into four main types:

- Adherens junctions (Zonula Adherens)
- Desmosomes (Macula Adherens)
- Gap junctions
- Tight junctions (Zonula Occludens)

Hemidesmosomes and *Focal Adhesions* – connect cells to the extracellular matrix.

Adherens Junctions (Zonula Adherens) (Fig. 16-11)

Adherens junctions are composed of **transmembrane cadherin proteins** and **catenin-associated** proteins, which attach through adherens junctions to the **actin** cytoskeleton. The cytosolic domains of cadherins bind to **β -catenin**, which attaches to **α -catenin**, a linker protein between the actin cytoskeleton and the adherens junction.

Disruption of adherens junctions in **radial glia** and **ependymal cells** has been linked to **hydrocephalus**, as it impairs the formation of a stable **ependymal layer**. This structural defect affects the **circulation of cerebrospinal fluid (CSF)**, leading to its accumulation and increased intracranial pressure.

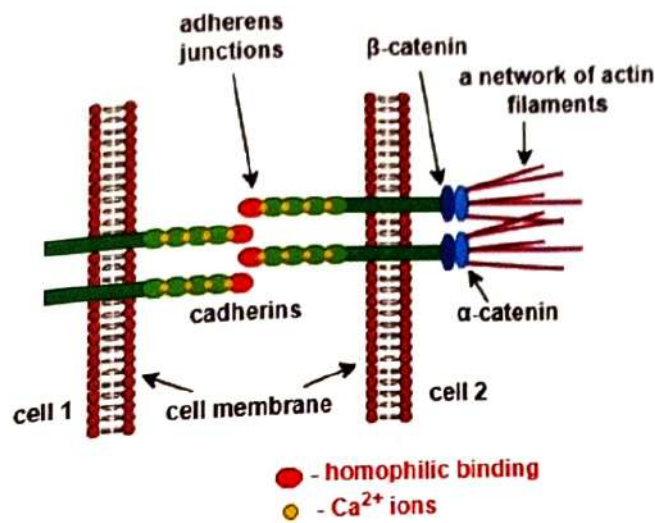


Fig. 16-11 Adherens junctions

Desmosomes (Macula Adherens) (Fig. 16-12)

Desmos = bridge

Desmosomes are **cell-cell adhesion structures** that provide **mechanical stability** in epithelial and cardiac muscle cells. They are composed of **intermediate filaments**, anchored to a **dense protein plaque** located beneath the plasma membrane. These structures serve both as **adhesive intercellular junctions** and as **cytoskeletal linkers**, maintaining the integrity of tissues exposed to mechanical stress. Desmosomes are formed by **desmosomal cadherins**, including **desmocollins (DSC1–3)** and **desmogleins (DSG1–3)**, which mediate **calcium-dependent adhesion**, similar to adherens junctions. The intracellular domains of these cadherins are linked to **intermediate filaments** via **desmoplakin**, a key cytoplasmic anchoring protein. Additionally, **plakoglobin** and **plakophilin** are present in the desmosomal plaque, further stabilizing the junction. During **cell transformation and tissue remodeling**, desmosomes may disassemble, altering cellular adhesion properties.

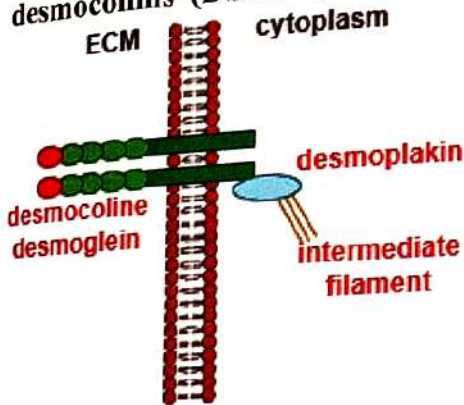
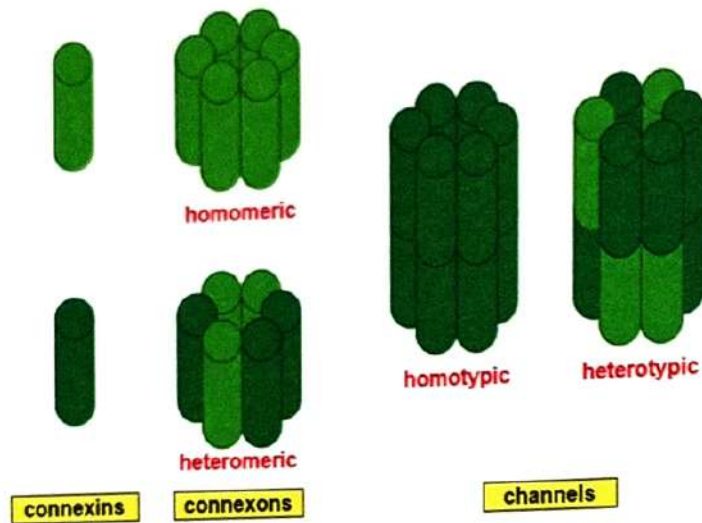


Fig. 16-12 Desmosomes

Clinical manifestations and pathohistology of desmosomal disorders

Desmosomes play a crucial role in maintaining **mechanically stable cell-cell adhesion**, particularly in tissues exposed to high mechanical stress, such as the skin and heart. Their importance is evident in **autoimmune disorders**, where antibodies target **desmosomal cadherins**, and in **genetic diseases** caused by mutations in desmosomal proteins, both of which lead to **tissue fragility and impaired adhesion**. One such autoimmune disorder is **pemphigus vulgaris**, characterized by the loss of intercellular adhesion between **basal and suprabasal keratinocytes**, resulting in **blistering and skin erosions** due to desmoglein autoantibodies. Another example is **palmoplantar keratoderma**, a genetic condition marked by **abnormal thickening of the stratum corneum**, leading to severe **hyperkeratosis on the palms and soles**.

Gap Junctions (Fig. 16-13)



Gap junctions function as **intercellular channels** and are involved in **signal transduction** between **adjacent cells**. They are permeable to **small molecules** (molecular weight <1 kDa), such as **nutrients, metabolites, ions, and small signaling transducers**. Communication via gap junctions is fundamental for coordinating cell behavior. Gap junctions are **highly reduced in most tumors**. Through them, apoptotic signals can spread to neighboring cells, leading to the destruction of cancer cells.

Fig. 16-13 Gap junctions

Lipid bilayers are crossed by protein complexes, called **connexons**, each formed by **six connexin subunits**. **Two connexons** connect across the intercellular gap, forming a continuous aqueous channel linking the two cells. Connexons can be **homomers or heteromers**, and intercellular channels can be **homotypic or heterotypic**. Connexins form hollow cylinders with a diameter of **1.5 nm**. In the **cardiac and smooth muscle**, they **facilitate** the integration of electrical and chemical activity into a single functional unit. They play an important role in embryonic development.

Tight Junctions (Fig. 16-14)

Tight junctions are involved in **forming impermeable barriers** and in **separating** the cell membrane of epithelial cells into basolateral and apical regions. The proteins **claudin** and **occludin** form the transmembrane part of the junction, while the cytoplasmic part is linked to the **microfilament network**.

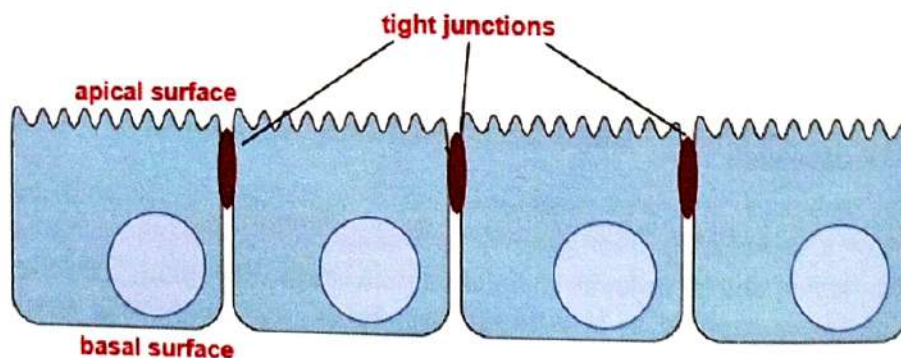


Fig. 16-14 Tight junctions

Hemidesmosomes and focal adhesions

Hemidesmosomes and focal adhesions are specialized cell-matrix adhesion structures in epithelial cells. **Hemidesmosomes** anchor cells to the basement membrane by connecting to the **intermediate filament network**, ensuring structural stability. In contrast, **focal adhesions** link the extracellular matrix (ECM) to the **actin cytoskeleton**, playing a key role in **cell migration and mechanosensing**.

Both structures contribute to essential cellular processes, including **attachment, proliferation, differentiation, and apoptosis**, highlighting their importance in tissue integrity and function.



17. Extracellular matrix (ECM)

The **function of connective tissue (CT)** is to **connect** individual cells into **tissues** and **organs**.
CT consists of:

I. Cells:

1. Synthesizing and secreting ECM components – fibroblasts, odontoblasts, ameloblasts, chondroblasts;
2. Remodeling ECM components – osteoclasts.

II. Extracellular matrix:

1. Structural proteins (collagen, elastin, fibrillin) and specialized proteins (fibronectin, laminin, etc.);
2. Glycoconjugates – glycoproteins (GP) and proteoglycans (PG);
3. Carbohydrate polymers – hyaluronic acid.

Biomedical significance of ECM

1. In embryonic development, ECM enables **cell migration**;
2. In acute and chronic **inflammation**, as well as in rheumatoid arthritis and osteoarthritis, ECM undergoes biochemical changes;
3. ECM plays a role in **tumor metastasis**;
4. Some diseases (osteogenesis imperfecta, different types of Ehlers-Danlos syndrome) are due to **genetic defects in collagen synthesis**;
5. Genetic defects causing lysosomal hydrolase deficiencies lead to impaired degradation of glycosaminoglycans (GAGs), resulting in mucopolysaccharidoses. These conditions involve pathological **accumulation** of GAGs in various tissues;
6. **Aging** also induces changes in ECM. Understanding these mechanisms allows cosmetics to develop agents that correct and slow undesirable ECM changes.

Collagen

1. Collagen is the **most abundant** protein in the human body – **25% of total proteins**; **30%** of the dry weight of bones; **50%** of the dry weight of cartilage. It is especially abundant in **skin, ligaments, tendons, bones, teeth, blood vessels, and internal organs**.
2. It represents a family of **28 different collagen types**, derived from **about 30** different polypeptide chains (PPC). Each collagen type is encoded by a **separate gene**.
3. The **primary structure** consists of **three PPC chains**, with amino acid (AA) sequences following the pattern **(Glycine-X-Y)_n**, where **X and Y** can be any AA, but are most often **proline** or **hydroxyproline (OH-proline)**, and less frequently **lysine** or **hydroxylysine (OH-lysine)**.

Classification of the main types of collagen:

1. Fibrillar collagens

- Type I (bones, tendons, dentin, lung, skin, muscles)
- Type II (cartilage, cornea, vitreous body)
- Type III (associated with Type I – lung, embryonic skin, vascular wall)
- Types V and XI (connective tissue)

2. Non-fibrillar collagens

- Types IV and VII – in ECM and basement membrane
- Types VIII and X – short chains, found in vascular endothelium and cornea
- Types IX, XII, and XIV – associated with fibrillar collagens

Collagen is a heterotrimer – $(\alpha 1)2\alpha 2$, with each chain containing 1,050 amino acids. Each PPC forms a left-handed α -helix, with three AAs per turn. Three collagen PPCs form a **right-handed superhelix**, known as **tropocollagen** (triple helix). Glycine is regularly positioned (every third AA is glycine) (Fig. 17-1). Glycine is a **small molecule**, allowing it to fit into the **central region** of the superhelix. Proline and OH-proline make up ~25% of the amino acids. If **OH-proline formation is impaired** (e.g., due to **vitamin C deficiency**), **unstable collagen** is produced.

Tropocollagen is stabilized by hydrogen bonds between the PPCs, as well as by **covalent cross-links** between **lysine** and **oxidized lysyl residues**.



Collagen is insoluble in water.

OH-lysine is essential for glycosylation.

Fig. 17-1 Collagen structure

Synthesis and post-translational modification (maturation) of collagen

Collagen is synthesized as a **high-molecular-weight precursor** (preprocollagen).

1. Intracellular stage of collagen synthesis:

- Insertion of preprocollagen molecules into the endoplasmic reticulum, facilitated by a **signal sequence**;
- *Proteolytic removal* of the signal sequence;
- *Hydroxylation of lysine* $\rightarrow$ 5-OH-lysine (*lysyl hydroxylase*, Fe^{2+} , vitamin C, O_2 , α -ketoglutarate);
- *Hydroxylation of proline* $\rightarrow$ 4-OH-proline or 3-OH-proline (*prolyl hydroxylase*, Fe^{2+} , vitamin C, O_2 , α -ketoglutarate) (see Fig. 2-4);

Thus, a **vitamin C deficiency** leads to **scurvy**. According to one theory of **rickets**, **vitamin C deficiency** blocks **bone and tooth formation**.

- *Glycosylation* of OH-lysine (with galactose, glucose, enzymes – *transferases*);
- Formation of **S-S bridges** at the N- and C-terminal non-collagenous regions of procollagen;
- *Triple helix formation*.

2. Extracellular stage of collagen synthesis:

- **Proteolytic removal** of the N- and C-terminals of tropocollagen by proteolytic enzymes (*procollagen aminopeptidase* and *procollagen carboxypeptidase*, collectively called *proteases*) (see Fig. 17-2);

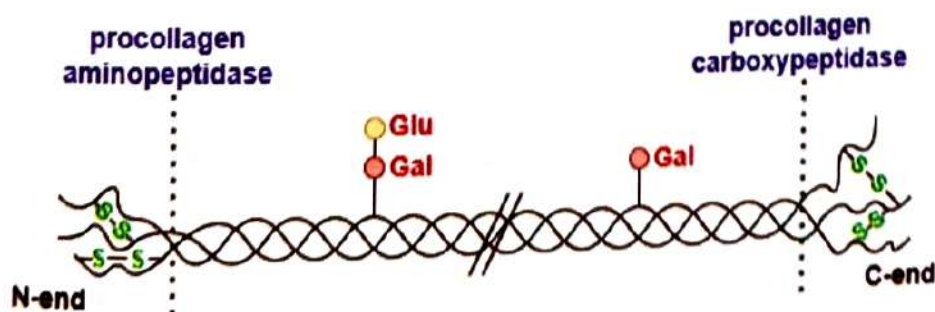


Fig. 17-2 Proteolytic removal of the N- and C-terminals of tropocollagen

- Organization of collagen fibrils and alignment in length (**microfibril, fibril, fiber**);
- **Oxidation of lysyl** (or OH-lysyl) residues in tropocollagen (fibrillar collagen) to **allysine** (lysyl aldehyde) by **lysyl oxidase** (Cu^{2+}) (see Fig. 17-3). This post-translational modification is essential for collagen and elastin synthesis, forming **highly reactive aldehydes**, which condense with other aldehydes or unmodified lysine residues to create various **cross-links**.

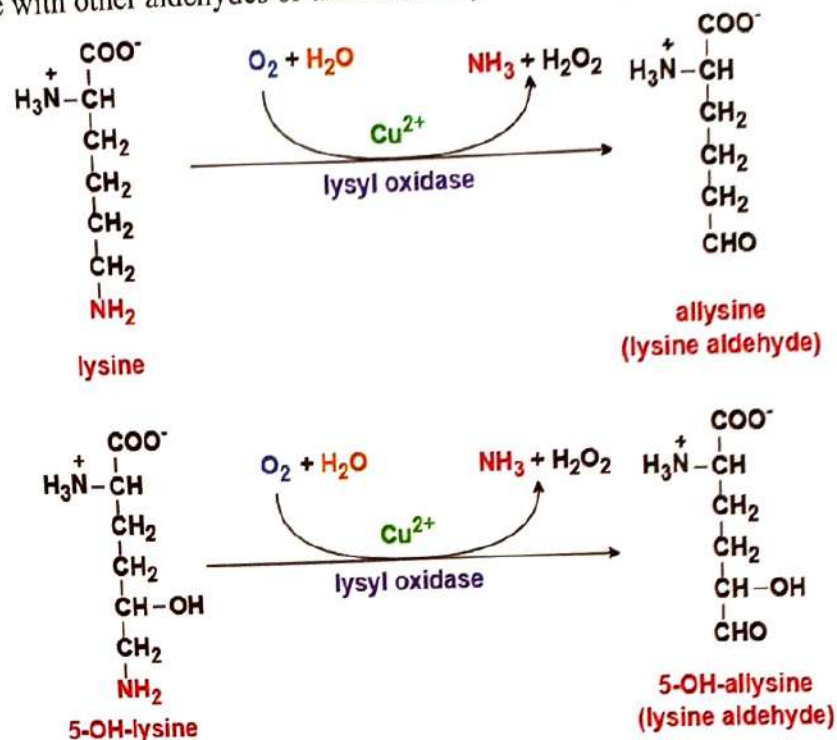


Fig. 17-3 Oxidation of lysine and OH-lysine

- Formation of inter - and intramolecular covalent bonds – **covalent cross-linking** (**spontaneous**):
 - Formation of **Schiff bases** followed by reduction of double bonds (see Fig. 17-4);
 - **Aldol condensation** (see Fig. 17-5).

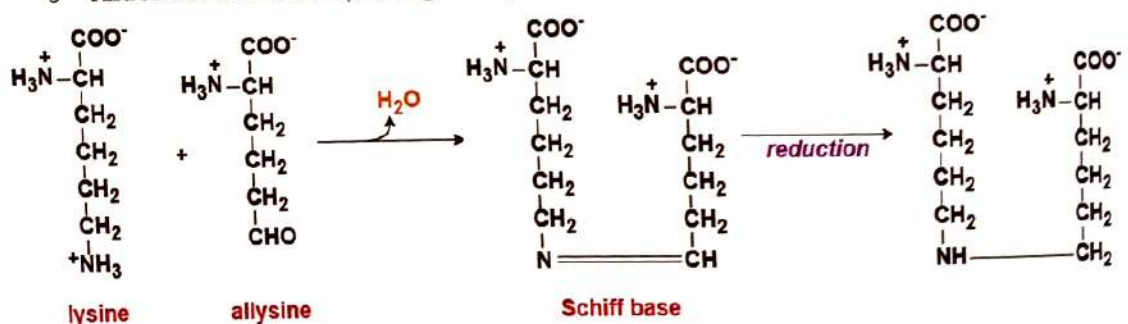


Fig. 17-4 Schiff base formation and double bond reduction

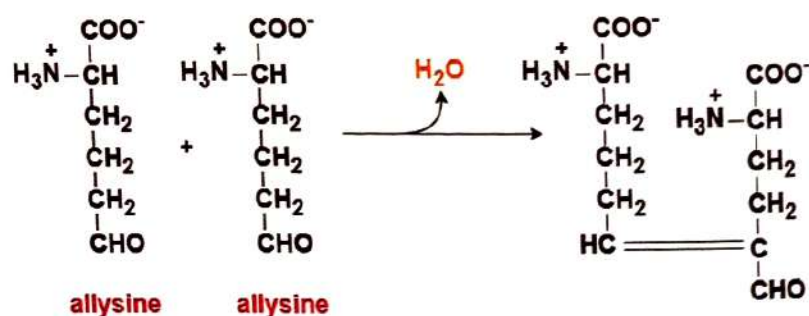


Fig. 17-5 Aldol condensation of allysine with another allysine molecule

- Formation of covalent peptide bonds by *transglutaminase* enzyme (Fig. 17-6) – intrachain or interchain.

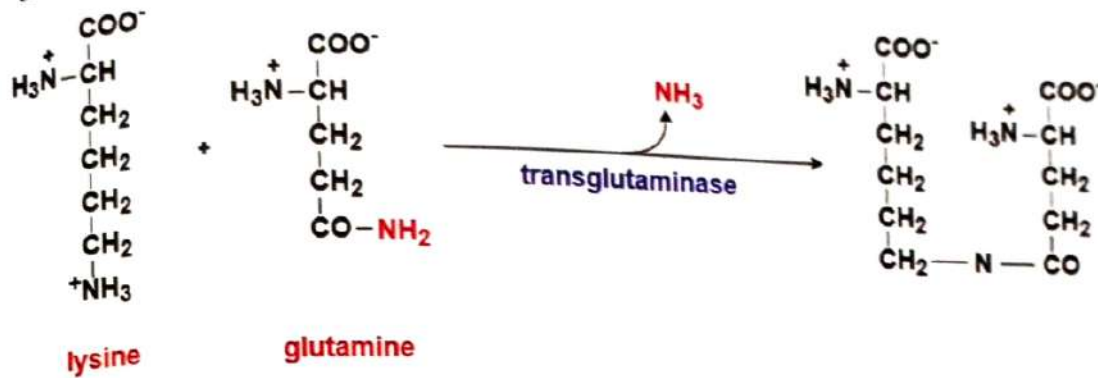
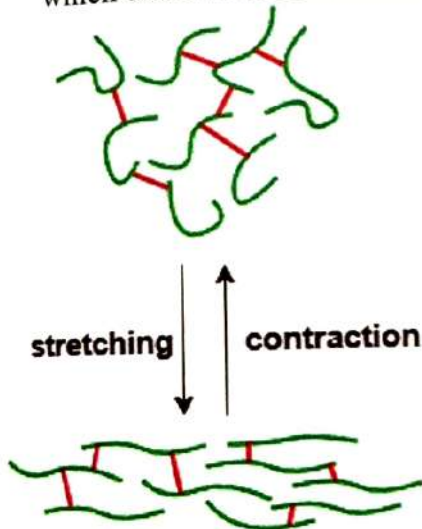


Fig. 17-6 Formation of a covalent peptide bond with the enzyme transglutaminase

Elastin

- Responsible for **stretchability** and **elasticity** (higher elasticity of elastin polypeptide chains, but also **returning** to their original position like a rubber band, unlike collagen, which **tears** when **overstretched**) in (Fig. 17-7):



- Lungs, large arterial vessels; elastic ligaments (menisci); less in skin, ear cartilage.
- One gene produces multiple elastin isoforms (unlike collagen types, which are encoded by separate genes);
- Structure: Synthesized as **soluble monomer** (tropoelastin); some proline residues are hydroxylated, but:
 - OH-proline is not glycosylated (not a glycoprotein);
 - Tropoelastin lacks extra N- and C-terminals and OH-lysine;
 - No regular (Gly-X-Y) sequence;
 - Does not form a triple helix.

Fig. 17-7 Contraction and stretching of elastin

Extracellular maturation of elastin – formation of desmosine and isodesmosine

- *Lysyl oxidase* catalyzes the oxidative deamination of lysine residues into **allysine**. These allysine molecules then participate in the formation of **inter- and intrachain covalent cross-links**. Specifically, **three** allysine molecules and **one** lysine residue form a unique tetrafunctional cross-link called **desmosine** (or **isodesmosine**), which is essential for the elasticity and structural stability of elastin.